Antimitotic Library
Small molecule compounds targeted against mitotic kinases

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 impressive 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. Proteins that control cellular progression through mitosis include kinases and cysteine proteases, namely Polo, Bub, Mad, Aurora, Cdk1, separase and others.

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](image-url)
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

<table>
<thead>
<tr>
<th>Kinases/ Family</th>
<th>Basic Function</th>
<th>Mitotic Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdk1/2</td>
<td>The serine-threonine kinases critical for controlling all phases of cell cycle progression</td>
<td>Multiple mitotic phases</td>
</tr>
<tr>
<td>(Cyclin-dependent kinase 1 and 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plk1</td>
<td>Regulate chromosome segregation and cytokinesis</td>
<td>Multiple mitotic phases</td>
</tr>
<tr>
<td>(Polo-like kinases)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bub1, BubR1 and TTK/Esk</td>
<td>Involved in chromosome segregation and kinetochore attachment</td>
<td>Methapase- Anaphase</td>
</tr>
<tr>
<td>Nek2/6/11</td>
<td>Control the centrosome structure during the mitotic cell cycle</td>
<td>Interphase-prometaphase, metaphase-anaphase</td>
</tr>
<tr>
<td>(NIMA kinases)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aurora A-C</td>
<td>Involved in chromosome separation, centrosome separation and cytokinesis</td>
<td>Multiple mitotic phases</td>
</tr>
<tr>
<td>(Aurora kinases)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEN/SIN kinases</td>
<td>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</td>
<td>Cytokinesis</td>
</tr>
<tr>
<td>MAP kinase</td>
<td>Cell cycle regulated. Many types of signal-transduction, activates p90RSK, which in tern activates Bub1 during Xenopus oocyte maturation.</td>
<td>Interphase and metaphase</td>
</tr>
<tr>
<td>(Mitogen-activated kinases)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mps1p</td>
<td>Implicated in the duplication of spindle pole body Recruits checkpoint proteins at kinetochores; Reported substrates include Mad1, Spc110</td>
<td>Interphase-prometaphase</td>
</tr>
<tr>
<td>(MonoPolar Spindle 1 kinase, Dual-specificity kinase)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wee1 and Myt1</td>
<td>Implicated in the DNA structure checkpoints</td>
<td>Interphase</td>
</tr>
<tr>
<td>Sid1p/2p</td>
<td>Sid kinases are components of the spindle pole body at all stages of the cell cycle and directly implicated in cytokinesis</td>
<td>Cytokinesis</td>
</tr>
<tr>
<td>(Sid kinases)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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.

As mentioned above, mitotic kinases are key players in mitotic checkpoints. Thus, CDK1 mitotic kinase, a nonredundant cyclin-dependent kinase (CDK), plays an essential role in the cell division, particularly during early mitotic events (entry 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)). 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. Notably, Plk1 kinase also activates Cdc25C.

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). 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). DNA replication and centrosome duplication are controlled by E2F transcription factors, Mps1p kinases, cyclin A/E and Cdc20 protein.
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)×.

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×. 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×ii. 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)×iii. 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. 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*

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Development phase</th>
<th>Type of action</th>
<th>Therapeutic area</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON-01910 1</td>
<td>Phase I</td>
<td>Plk-1 inhibitor</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>ON-1910Na 2</td>
<td>Phase I</td>
<td>Plk-1 inhibitor with IC₅₀ = 9 nM</td>
<td>Leukemia and solid tumors therapy</td>
</tr>
<tr>
<td>BI-2536 3</td>
<td>Phase I</td>
<td>Plk-1 inhibitor</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>Wortmannin 4</td>
<td>Preclinical</td>
<td>Plks inhibitor</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>Scytonemin 5</td>
<td>Preclinical</td>
<td>Plk-1 inhibitor with IC₅₀=2.3-3.4 µM</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>β-Hydroxyisovalerylshikonin (β-HIVS) 6</td>
<td>Preclinical</td>
<td>Plk-1 inhibitor</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>Staurosporine 7</td>
<td>Preclinical</td>
<td>Plk-1 inhibitor with IC₅₀ = 0.8±0.2 µM</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>VX-680 8</td>
<td>Phase II</td>
<td>Aurora A, B and C inhibitor in vitro with IC₅₀ values of 0.6, 18 and 4.6 nM, respectively</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>MLN-8054 9</td>
<td>Phase I</td>
<td>Aurora A kinase inhibitor</td>
<td>Solid tumors therapy</td>
</tr>
<tr>
<td>PHA-680632 10</td>
<td>Preclinical</td>
<td>Aurora A and Aurora B inhibitor (IC₅₀ = 27 and 135 nM, respectively)</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>PHA-739358 11</td>
<td>Phase II</td>
<td>Aurora-A,B and C inhibitor</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>AZD-1152 12</td>
<td>Phase I</td>
<td>Aurora-B and C inhibitor</td>
<td>Hematological cancer and solid tumors therapy</td>
</tr>
<tr>
<td>VX-528**</td>
<td>Preclinical</td>
<td>Aurora-B (ARK2) kinase inhibitor</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>ZM-447439 13</td>
<td>Preclinical</td>
<td>Aurora A and B kinases inhibitor with IC₅₀ values of approximately 0.1 µM</td>
<td>Cancer therapy</td>
</tr>
<tr>
<td>MP-235 14</td>
<td>Preclinical</td>
<td>Aurora kinases A,B and C with an IC₅₀ of 90 nM</td>
<td>Cancer therapy (antiproliferative effects in cancer cell)</td>
</tr>
</tbody>
</table>
**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\textsuperscript{xv}. For example, they are essential for the activity of the MT organization center\textsuperscript{xvi}. They are important players in mitotic entry, spindle formation and cytokinesis\textsuperscript{xvii}. 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\textsuperscript{xviii}. 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\textsuperscript{xix} (Fig. 4).
Inhibitors of Aurora kinases

Serine/threonine protein kinases of Aurora family are involved in chromosome segregation and cell division in all eukaryotes. They were first identified in the cell cycle studies as *Xenopus* Eg2. These enzymes are essential in the “spindle checkpoint” system used by cells to monitor fidelity of mitosis. 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. 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. Aurora A is localized to centrosomes from S/early G2 phases. It is required to establish a bipolar mitotic spindle. 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. 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. Further studies suggested that they play pivotal role in development of breast, colorectal, bladder and ovarian cancers (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.

<table>
<thead>
<tr>
<th>Aurora kinase</th>
<th>Cell lines/human cancers</th>
<th>Overexpression/amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aurora-A</strong></td>
<td>Breast cancer cell lines</td>
<td>30-40%</td>
</tr>
<tr>
<td></td>
<td>Ductal invasive carcinomas</td>
<td>94%</td>
</tr>
<tr>
<td></td>
<td>Primary invasive breast cancers</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td>Node-negative breast carcinomas</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>Primary breast carcinomas</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>Primary colorectal cancers</td>
<td>&gt;50%</td>
</tr>
<tr>
<td></td>
<td>Ovarian cancer cell lines</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td>Sporadic ovarian cancers</td>
<td>44-54%</td>
</tr>
<tr>
<td></td>
<td>Hereditary ovarian cancers</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Aurora-B</strong></td>
<td>Colorectal cancer cell lines</td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>Primary human colorectal cancers</td>
<td>ND*</td>
</tr>
<tr>
<td><strong>Aurora-C</strong></td>
<td>Breast cancer cell lines</td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma cell lines</td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>Primary human colorectal cancers</td>
<td>52%</td>
</tr>
</tbody>
</table>

* 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\textsuperscript{xix}. Similarly, misregulated levels of Aurora-B yield abnormalities in chromosome attachment or alignment to the mitotic spindle during cellular mitosis\textsuperscript{xx}. Aurora-B may form complexes with Survivin, anti-apoptotic protein that is commonly overexpressed in tumors\textsuperscript{xxxi}. 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\textsuperscript{xxxi}. 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. 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).

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 kinase 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 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(Kd)) (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.
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.

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 more than 5000 small molecule compounds Representative set of Mitotic kinase-biased compounds is shown below.
Examples of compounds from the Mitotic kinase-targeted library (5000 cmpds)

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. 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. Nek2 kinase (NIMA family) promotes centrosome separation and may control histone H3 phosphorylation. MAP kinases regulate spindle dynamics and chromosome movement. Mad (1p, 2p and 3p) proteins are involved in a spindle assembly checkpoint mechanism. Mitotic kinases are critical for the mitotic exit and cytokinesis. 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 more than 5000 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.
Small molecule compounds targeted against mitotic kinesins and separases

Introduction

Mitosis is a critical stage in the cell cycle. Newly replicated chromosomes are segregated to opposite poles of a dividing cell. This step is followed by cell division to yield two daughter cells. Inhibition of mitosis is a powerful strategy for treating diseases associated with excessive cell proliferation.\textsuperscript{xlvii} In particular, antimitotic drugs have been the most successful pharmacological agents for the treatment of numerous tumors.\textsuperscript{xlviii}

Mitotic spindle is the central component of mitosis. It is primary target for the development of antimitotic agents.\textsuperscript{xlix} Mitotic spindle is responsible for the separation of replicating chromosomes into daughter nuclei during division of nucleus (karyokinesis). Furthermore, it directs the process of dividing the cytoplasm (cytokinesis). Once spindle formation is blocked (e.g., by treatment of a drug), chromosomes condense but do not follow through the normal mitosis and division stops. In other words, mitotic spindle represents cellular machinery that converts chemical energy into mechanical work needed to move chromosomes and divide cell. The main entities regulating formation and dynamics of mitotic spindle include microtubules (MTs), motor proteins and mitotic checkpoint enzymes.\textsuperscript{l}

2. Limitations of the direct tubulin-targeted therapy

Despite utility of taxanes and vinca alkaloids in clinic, there are serious limitations to these therapies.\textsuperscript{li} On-target toxicity of these agents is associated with the notion that tubulin polymers play critical role in the non-mitotic cytoskeletal functions in both proliferating and terminally differentiated cells. Microtubules are also essential for axonal transport in neurons.\textsuperscript{lii} Peripheral neurotoxicity of Paclitaxel\textsuperscript{TM}, Docetaxel\textsuperscript{TM} and Vincristine\textsuperscript{TM} has been extensively studied.\textsuperscript{liii} Although manageable and reversible for the majority of second generation anti-mitotic drugs, this severe side effect may preclude repeated courses of therapy. Neuropathy continues to be an issue for novel agents in clinical development, for example Dolastatin-10.\textsuperscript{lv}

Another significant limitation of current modulators of tubulin dynamics is their marginal clinical efficacy. While demonstrating impressive \textit{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. For example, vinca alkaloids and taxanes are both substrates for the P-gp efflux pump encoded by the multidrug resistance \textit{mdr1} gene, resulting in decreased sensitivity to these compounds \textit{in vivo}.\textsuperscript{lv} Typical MT-targeting drugs exert
anti-mitotic effects by changing spindle microtubule dynamics. Alterations in a total tubulin or tubulin isotype content furnish compensatory effects. For example, changes in tubulin expression\textsuperscript{li} and mutations in β-tubulin can affect Paclitaxel\textsuperscript{TM} driven polymerization.\textsuperscript{lii} Therefore, 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.

3. Novel non-tubulin mitotic targets and their small-molecule inhibitors

Due to these limitations of the tubulin-binding antimitotic agents, there is ongoing need to identify other targets that yield anti-mitotic effect with better specificity and more predictable pharmacology. 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.\textsuperscript{liii} Proteins that control cellular progression through mitosis include kinases and cysteine proteases, namely Polo, Bub, Mad, Aurora, Cdk1, separase and others.\textsuperscript{lix} The significant role of mitotic kinases was partly elucidated within the description (Part I) which supports the ChemDiv antimitotic library. Here we provide the CL consisted solely of mitotic kinesin- and separase-targeted agents.

4. A brief introduction in the mitotic kinesins structure and functions

The formation and proper function of the mitotic spindle depend on the dynamic properties of MT and function of MT-dependent motor proteins. Motor proteins can be defined as molecular machines that convert chemical energy of ATP hydrolysis into mechanical work used to power cellular motility (i.e. force and motion).\textsuperscript{lx} For example, motors can crosslink and slide overlapping antiparallel MTs within interpolar MT bundles to position spindle poles in relation to one another. Mitotic motors reportedly use at least three distinct mechanisms to perform their function.\textsuperscript{lix} These are: (1) crossbridging and sliding MTs relative to adjacent MTs or other structures; (2) transporting specific mitotic cargoes along the surface lattice of spindle MTs; and (3) regulating MT assembly dynamics and coupling movement to MT growth and shrinkage. Specific mitotic movements are not driven by individual motors. Instead they are a result of shifts in dynamic balance of complementary and antagonistic forces generated by multiple motors functioning cooperatively. Motor proteins are involved in organizing MTs into a spindle and in moving chromosomes. Some motor proteins play direct role in assembling the spindle and linking its components into a coherent unit. Others are responsible for attaching chromosomes to the spindle and cortex generating forces for their motions. The unremitting toil of motor proteins is observed along microtubules between the poles and the chromosomes, at the kinetochores and along the arms of the chromosomes. Many types of motors are detected in a single location, whereas some of them are found in several different places.
There are more than 45 different kinesins in humans. They are separated into three major classes according to the motor domain localization and their amino acid sequence. Kinesins with N-terminal motor domains (“+” directed motors) move predominantly towards the plus end of microtubules. Kinesins with C-terminal motor domains (“−” directed motors) migrate towards the minus ends. Kinesins with centrally-located motor domains destabilize ends of MTs. Outside the motor domains, kinesins are quite divergent. Majority of these proteins are homodimers with identical ATP-catalytic cores (heads). Each head is connected to the “necklinker,” a mechanical element that undergoes nucleotide-dependent conformational changes that enable motor stepping. The mechanistic pathway of kinesin movement was detected using a FIONA (Fluorescence Imaging One-Nanometer Accuracy) protocol. Using this technique, two models of kinesin movement have been postulated: the hand-over-hand “walking” model in which the two heads alternate in the lead (Fig. 1A), and an inchworm model in which one head always leads (Fig. 1B).

Mitotic kinesin activity is essential for the formation and function of the mitotic spindle, chromosome segregation and transport, mitotic checkpoint control, and cytokinesis. These roles have been extensively studied in Saccharomyces cerevisiae and Drosophila cells. The yeast, Saccharomyces cerevisiae contains two members of bipolar mitotic kinesin family, Cin8p and Kip1p. Following the seminal discovery of two kinesin motors with mitotic functions it has become established that a strikingly large number of kinesin-like motors participate in a spindle action and mitosis. Today, the kinesin superfamily as a whole is large and diverse with hundreds of sequences that can be grouped into 17 major families based on sequence similarity and main functions. Current summary of roles that kinesins play in cell division is summarized in Table 1.

Table 1. Published functional data for kinesins with critical roles in the cell division
Mitosis is a set of ordered mechanical events (I-IV): I, microtubule capture and spindle pole separation; II, chromosome alignment; III, anaphase chromosome movement; IV, telophase and cytokinesis (Fig. 2A). During mitosis, kinesin-like motors perform several basic functions illustrated within Fig. 2B. Some of them, such as Kif2A and highly conserved role in spindle pole focusing and spindle length determination. In eukaryotes, this protein localizes to spindle poles and spindle microtubules. Electron microscopy suggests that HSET localizes between microtubules in parallel bundles. HSET prevents two opposing MT arrays from separating by opposing pushing force of Eg5. Functions of kinesins during all mitotic stages have been fairly described in recent review article.
5. Small molecule inhibitors of mitotic kinesins

As briefly discussed above, tubulin binding agents feature serious side effects related to the universal role of tubulin in important cellular processes such as the maintenance of organelles and cell shape, cell motility, synaptic vesicles, and intracellular transport phenomena. In addition, application of tubulin-binding agents is limited by drug resistance. From this point of view, small molecule inhibitors of mitotic kinesins represent promising class of anticancer drugs. In the past few years a number of kinesin-targeting agents were found. Several inhibitors are currently in clinical developed as anti-cancer agents (Table 2, Fig. 3).

Table 2. Small-molecule inhibitors of kinesin-like proteins in preclinical and clinical development*

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**Fig. 2.** (A) Kinesin-related proteins as important modulators of cell division; (B) specific functions of Kinesin-like motors

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As shown in table 2, vast majority of small molecules target kinesin KSP. Inhibition of KSP led to the cell cycle arrest during mitosis, yielding cells with monopolar spindles. In contrast to classical mitotic inhibitors (Vinca alkaloids and Taxanes), KSP inhibitors did not interfere with other microtubule-dependent processes. The first specific KSP inhibitor was Monastrol, (4-aryl-3,4-dihydro-pyrimidine-2(1H)-thione) (1) (Fig. 3). It is an allosteric inhibitor of KSP with an IC₅₀ value of 14-34 μM. Several new analogues were recently synthesized and tested for anticancer activity, for example enastron, enastrol, VS-83 and others.
6. Separase as potential antimitotic target

Duplication of chromosomes into sister chromatids is one of the critical stages in mitosis. During anaphase, chromatid separation is meticulously controlled by many specific proteins. Errors associated with this process cause instability in the transmission of inherited material resulting in death, aneuploidies, and formation of cells with supernumerous or missing chromosomes. The final irreversible step in the duplication and distribution of genomes to daughter cells takes place when chromosomes split at the metaphase-to-anaphase transition. Various aspects of chromosome segregation are closely related to a cell cycle control including proteolysis and stage-specific protein modification. Several
proteins are important in this process. These include SMC-containing cohesin and condensin, APC/C ubiquitin ligase, Aurora kinases, DNA topoisomerase II, securin-separase complex and kinetochore microtubule destabilizers or regulators. Symmetrical segregation of sister chromatids during anaphase depends mainly on cohesion regulated by a complex of chromosomal proteins called cohesin. It is believed to hold replicated sister DNA strands together after their synthesis. This allows pairs of replication products to be recognised by the mitotic spindle mechanism in mitosis for segregation into opposite direction. Separase is a critical protease that triggers chromatid disjunction at anaphase onset by cleaving cohesin’s subunit (Fig. 4).

During mitosis in vertebrates, cohesin is removed from chromosomes via two mechanisms. First mechanism involves Plk-1 and Aurora B kinases. They phosphorylate Sec3 subunit of cohesin located in terminal parts of the sister chromosome complex. However, a small centromeric fraction of cohesin sustains pairing until the end of metaphase. This protein complex consists of protein phosphatase 2A (PP2A) and shugoshin. According to the second mechanism, sister chromatid separation is mediated by separase. It cleaves Scc1/Rad21 subunit of remaining centromeric cohesin. During earlier mitotic stages, separase is held inactive by association with securin. During metaphase-anaphase transition, securin is degraded via ubiquitin-proteasome pathway. Homozygous deletion of securin in a human cancer cell lines reportedly caused massive chromosome missegregation. Notably, securin knockout mice exhibited viability and surprisingly mild phenotypes suggesting the existence of additional securing-independent mechanism of separase regulation. Small molecule inhibitors of separase could be of interest as anti-tumor agents as this protease is a key player in cohesin dissociation and sister chromatids separation.

In addition, it was also found that vertebrate separase acts as a Cdk1 inhibitor. For example, wild type separase but not a phosphorylation site mutant compromised in Cdk1 binding can overcome a block of mitotic exit imposed by non-degradable cyclin B1. Thus, in the separase-Cdk1 complex the inhibition is mutual - not only does Cdk1 inhibit the protease but at the same time separase inactivates...
the kinase. The mutually exclusive binding of securin and Cdk1 makes separase an unusually regulated Cdk1 inhibitor, which becomes active only upon removal of securin.

Concept and Applications

AM-library design at CDL involves:

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

1. Unique morphing and funneling procedures in designing novel potential mitotic kinesins-targeted ligands 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.

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 (T1/2), Volume of distribution in human plasma (Vd), 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; SAR is implemented in the next library generation.

We practice a multi-filter approach for building AM-focused library:

Self-organizing Kohonen maps belong to a class of neural networks known as competitive learning or self-organizing networks which in turn are based on unsupervised learning rule (see notes). They were originally developed to model the ability of the brain to store complex information as a reduced set of salient facts without loss of information about their interrelationships. High-dimensional data are mapped onto a two-dimensional rectangular or hexagonal lattice of neurons in such a way as to preserve the topology of the original space. This methodology has successfully been used in various medicinal chemistry applications.

We have used this approach for compound selection and focused-library profiling. Initially, we
have collected a 22,110-compound database of known drugs and compounds entered into preclinical or clinical trials; their structures and assignments were obtained from Prous Science Integrity [Prous Science, URL: http://www.prous.com]. Each compound within this database was characterized by a defined profile of target-specific activity, focused against 1 of more than 100 different protein targets. In particular, this set included more than 600 kinesin-targeted agents which were shown to have activity against mitotic kinesin-like proteins as well as separase; representative structures are shown in Fig. 4. The whole dataset was then filtered and preprocessed. It was filtered based on MW (not more than 800). Molecular features encoding the relevant physicochemical and topological properties of compounds were then calculated using SmartMining software [URL: http://www.ChemDiv.com] 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. As a result of specific selection procedure, at the output, an experimental set consisted of 7 molecular descriptors including Zagreb index, E-state indexes for the following structural fragments: >C-, -CH2-, -CH3, the number of H-bond donors, HB2 (a structural descriptor which encodes the strength of H-bond acceptors following an empirical rule) and LogP was determined. This set was then used for Kohonen map generation. 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. However, it should be noted that for each particular target-specific activity group, another, more optimal set of descriptors can be found, which provides better classification ability.

A Kohonen SOM of 22K pharmaceutical leads and drugs generated as a result of the unsupervised learning procedure is depicted in Fig. 5. It shows that the studied compounds occupy a wide area on the map, which can be characterized as the area of druglikeness. Distribution of various target-specific groups of ligands in the Kohonen map demonstrates that most of these groups have distinct locations in specific regions of the map (Fig. 6a-f). As shown in Fig 6a, pharmaceutically relevant agents targeted against mitotic kinesins occupy compact areas within the map constructed. The classification accuracy of Kohonen modeling was approx. 77%. It is a statistically relevant prediction quality therefore this model can be effectively used for virtual library profiling of the current interest.

A possible explanation of these differences is in 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.
The described algorithm represents an effective procedure for selection of target-focused compound subsets compatible with high throughput in silico evaluation of large virtual chemical space. Whenever a large enough set of active ligands is available for a particular receptor, the quantitative discrimination function can be generated allowing selection of a series of compounds to be assayed against the target. It is important to note that focusing on physicochemical rather than structural features makes this approach complementary to any available ligand structure similarity technique.

A series of consecutive funneling procedures were also applied to enhance the target-specific relevance of novel compounds. During this step, we have addressed the compound’s lead-likeness (enforcing partial Rule of 3 compliance), the availability of unique R-groups, the pre-synthetic analysis
of privileged templates, the IP potential, the feasibility of high-throughput chemistry. We have also considered the ADME/Tox issues (such as HIA and BBB-permeability, plasma protein binding, cytochrome P450 substrate and inhibition potential) and key physico-chemical properties (such as DMSO and water solubility, stability and ionization potential, etc.). The funneling procedures were carried out by kinesin- and separase-specific neural networks (backpropagation NN, Kohonen- and Sammon-based SOMs), fragment and property-based models. Diversity of the final selection was optimized using proprietary algorithms.

**Synthesis and biological evaluation**

- Novel AM-targeted libraries are synthesized according to the above criteria.
- The subsets of AM-library 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 AM-focused library consisted of about 10,000 compounds targeted particularly against mitotic kinesins. Representative set of AM-biased compounds is shown in Fig. 7.

![Fig. 7. Examples of compounds from the AC-targeted library (Kinesin-targeted compounds)](image)

We provide rapid and efficient tools for follow-up chemistry on discovered hits, including single isomer chemistry, stereoselective synthesis and racemic mixture separation. Targeted library is updated
quarterly based on a “cache” principle. Older scaffolds/compounds are replaced by templates resulting from our in-house development (unique chemistry, literature data, computational approaches) while the overall size of the library remains the same (ca. 10K compounds). 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.

Conclusion

Among a wide number of anticancer drugs which can be found on the pharmaceutical market as well as numerous compounds currently being evaluated in different preclinical and clinical trials, modulators of microtubule dynamics seems to remain the most important class of antimitotic 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. These issues led to identification of an alternative targets and signaling mechanisms that yield anti-mitotic effect with greater specificity and more predictable pharmacology. This category includes mitotic kinases (see the AM-description, Part I), mitotic kinesins and separase described herein.

Mitotic kinesins modulate the mitotic spindle by generating forces along both polar axes of microtubules and by actively regulating microtubule polymerization dynamics. Small molecules that selectively mediate kinesins may prove to be superior to the existing tubulin-targeting drugs by providing a wider efficacy-toxicity window. The anticancer effect of kinesin inhibitors may be augmented by their activity against endothelial cell proliferation, resulting in the inhibition of angiogenesis. Finally, inhibitors of mitotic kinesins may find broader application for the treatment of other proliferative diseases such as diabetic retinopathy, restenosis, pulmonary and liver fibrosis, lupus erythrematosus, and lymphoproliferative disorders that develop in patients with a history of autoimmune disease.

Separase is a large molecular weight protease that specifically cleaves the subunit Scc1/Rad21 of the cohesin complex thus maintaining the mitotic exit and chromatid separation. In spite of an obvious interest towards this promising biological target, currently, a relatively small number of therapeutically relevant agents are developed. Several compounds have been evaluated in different preclinical trials, some of them are now being regarded as potential drug-candidates, and their first-stage clinical evaluation will be coming soon. In the present approach to our AM-focused library profiling we have used the same Kohonen map to select small molecule compounds directly fallen into the nodes (see Fig. 7c) which were doubtless classified as areas of protease inhibitors (the predictive accuracy is approx. 80%). In should also be noted that this area is generally represented by aspartic protease inhibitors.
Integrally, the developed Kohonen-based computational model constructed for 22K pharmaceutically relevant agents represents a powerful tool for the prediction of kinesin inhibitory activity. It can also be effectively used for selecting the compounds which can be tentatively regarded as potential separase inhibitors. Thus, we have been effectively used this model to recruit about 12,000 small molecule compounds in our AM-focused library.

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