DOI: 10.1002/cmdc.200500045

Inhibitors of Mitotic Kinesins: Next-Generation Antimitotics

Vasiliki Sarli* and Athanassios Giannis*[a]

Introduction

A large number of antimitotics have been proven so far as valuable and effective anticancer agents in clinical oncology.[1] For example, the vinca alkaloids vincristine and vinblastine are used for the treatment of leukemia and Hodgkin's lymphoma, whereas paclitaxel and docetaxel are approved for the treatment of metastatic breast cancer, as well as for lung and ovarian carcinomas.[2] These drugs inhibit mitosis by targeting the spindle microtubules, which are responsible for cell proliferation and the faithful partitioning of genetic material.[3] However, despite their success, these antimitotics show several serious side effects such as neurotoxicity.[4] These side effects are related to the broad role of tubulin (the fundamental building-block protein of microtubules) in important cellular processes such as the maintenance of organelles and cell shape, cell motility, synaptic vesicles, and intracellular transport phenomena. A further limitation to the application of antimitotics is the development of resistance. Resistance mechanisms involve the multidrug-resistant phenotype[5] mediated by the P-glycoprotein efflux pump, tubulin mutations, alterations in the expression of tubulin isotypes, and microtubule-associated proteins.[6,7]

Lately, the revolution in molecular biology combined with advances in high-throughput screening and automated microscopy and imaging has yielded a better understanding of cell division, and has helped in the identification of new and more selective anticancer targets, including members of several kinase families such as Polo,[8] Aurora,[9] and mitotic kinesins. These proteins are exclusively involved in the formation and function of the mitotic spindle and are key regulators for mitotic entry, progression, and cytokinesis.

Mitotic Kinesins

Kinesins are motor proteins that use ATP energy to move along microtubules and are involved in cargo transport and mitosis.[10,11] There are more than 45 different kinesins in humans, and they are separated into three major classes according to where the motor domain is localized within their amino acid sequence.[12] Kinesins with N-terminal motor domains move predominately toward the plus end of microtubules, and kinesins with C-terminal motor domains migrate toward the minus ends; kinesins with centrally-located motor domains are specialized for the destabilization of microtubule ends.

Mitotic kinesin activity is essential for the formation and function of the mitotic spindle, chromosome segregation and transport, mitotic checkpoint control, and cytokinesis.[13] These roles have been extensively studied in *Saccharomyces cerevisiae*[14] and in *Drosophila* cells,[15] while recently the analysis of human motor proteins by RNA interference was examined. These investigations demonstrated the presence of at least 12 kinesins involved in mitosis and cytokinesis (Table 1).[16]

Table 1. Various kinesins and their functions.

Kinesin	Family	Function
Kif14	Kinesin-3	chromosome congression and alignment
Kif4A	Kinesin-4	midbody formation cytokinesis
Kif4B	Kinesin-4	midbody formation cytokinesis
Eg5	Kinesin-5	centrosome separation spindle bipolarity
MKLP1	Kinesin-6	midbody formation cytokinesis
MKLP2	Kinesin-6	midbody formation cytokinesis
CENP-E	Kinesin-7	chromosome congression and alignment
Kif18	Kinesin-8	chromosome congression and alignment
Kid	Kinesin-10	chromosome congression and alignment
Kif2A	Kinesin-13	spindle bipolarity
KifC1	Kinesin-13	spindle bipolarity chromosome congression and alignment
MCAK	Kinesin-13	chromosome congression and alignment

At the onset of mitosis, after centrosome duplication and DNA replication during interphase, the initial spindle assembly starts with the separation of centrosomes, which become the centers of microtubule organization (Figure 1). Experiments using RNA interference revealed that three kinesins, Eg5, KifC1, and Kif2A, are responsible for bipolar spindle assembly.[16] *Homo sapiens* Eg5 (KSP) is a slow plus-end-directed motor of the Kinesin-5 family (BimC family)[17] which localizes along the

[a] *Dr. V. Sarli, Prof. Dr. A. Giannis*
University of Leipzig
Institute for Organic Chemistry
Johannisallee 29, 04103, Leipzig (Germany)
Fax: (+49) 341-97-36-599
E-mail: giannis@chemie.uni-leipzig.de
sarli@chemie.uni-leipzig.de

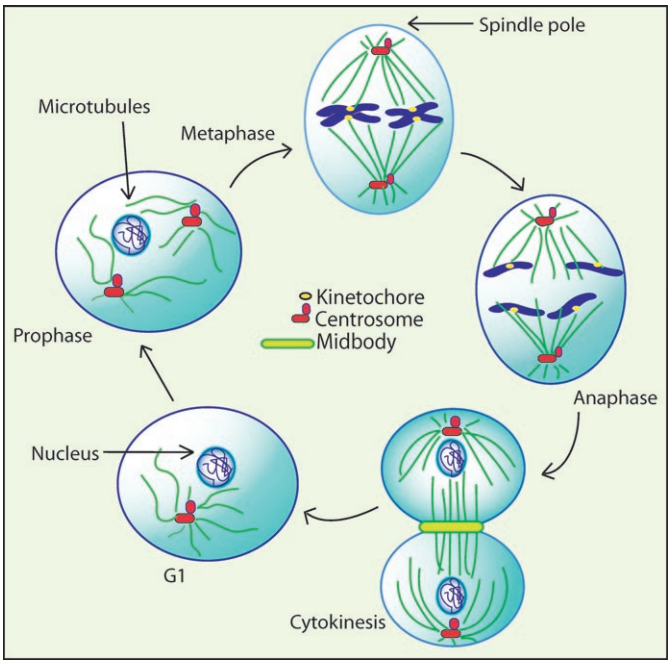


Figure 1. Subcellular components involved in the phases of cell division.

interpolar spindle microtubules and spindle poles.[18] This kinesin slides along the microtubules and creates forces for bipolar spindle formation and centrosome separation. BimC kinesins have a catalytic domain at the N-terminus, an α-helical coiled-coil stalk domain in the middle, a tail domain at the C-terminus, and they share a unique homotetrameric structure.[19–21] In contrast, KifC1 is a minus-end-directed motor with a cross-linking function that acts antagonistically toward the activity of the plus-end-directed motor Eg5 for spindle formation and stability.[22] Kif2A is an enzyme involved in depolymerization; depletion of Kif2A inhibits cell-cycle progression through the formation of monopolar spindles.[23]

During pro-metaphase, the condensed chromosomes begin to attach to the microtubules and move to the center of the spindle. When the chromosomes are correctly aligned at the metaphase plate, the spindle checkpoint is satisfied and anaphase starts with sister chromatid separation and chromosome segregation. Various kinesins orchestrate their duties and interact with kinetochores, microtubules, and chromosomes to perform the above functions. For example, the mitotic centromere-associated kinesin (MCAK), a depolymerizing kinesin, participates in the reordering of the microtubule cytoskeleton and is vital for the proper segregation of chromosomes.[24] The centromere-associated protein E (CENP-E) is essential for stable, bi-oriented attachment of chromosomes to spindle microtubules for the development of tension across aligned chromosomes and for the stabilization of spindle poles.[25] The chromokinesin Kid, a plus-end-directed motor, localizes on spindles and chromosomes and is required for the proper alignment of chromosomes along the metaphase plate.[26]

At the end of mitosis, the spindle elongates and the two daughter cells are separated during cytokinesis. Kinesins Kif4A and Kif4B,[16,27] as well as MKLP1[28] and MKLP2[29] are involved in midzone formation and play important and distinct functions in cytokinesis.

Taken together, it is becoming clear that kinesins are key players in the mechanisms of the spindle during mitosis and cytokinesis, and their inhibition is an attractive idea for the development of new anticancer strategies.[1] There are now several reports of small-molecule inhibitors of the kinesin Eg5, and studies of the first MCAK inhibitors were recently published.[30]

Eg5 Inhibition

The first Eg5-specific inhibitor identified was monastrol **1** (Figure 2), which arrests cells in mitosis with the formation of monoastral spindles (Figure 3).[31] The phenotype-based screening that was used for its identification opened a new way for the discovery of small molecules that affect mitotic spindle formation through a mechanism different from that of the known antimitotics.[32] Both the *R* and *S* enantiomers of **1** show inhibitory effects, but the *S* enantiomer appeared to be more potent and induces monoasters at lower concentrations than the *R* enantiomer or the racemic mixture.[33] Monastrol inhibits Eg5 motility with an IC_{50} value of 14 μM and causes a specific and reversible cell-cycle block.[31] Furthermore, monastrol is an allosteric inhibitor that binds to an induced-fit pocket 12 Å away from the catalytic center of the enzyme, as was determined by the crystallographic structure of the human Eg5 motor domain in complex with Mg^{2+}, ADP, and monastrol.[34]

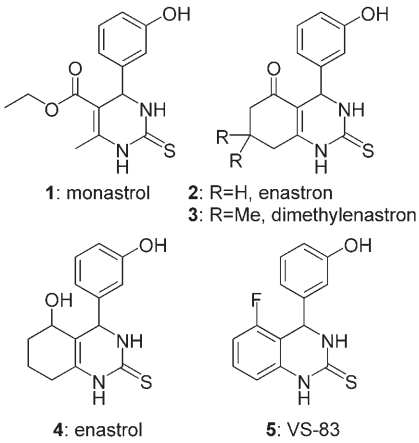


Figure 2. Monastrol and monastrol analogues.

Recently, several monastrol analogues were synthesized and studied for their ability to inhibit Eg5 (Figure 2). Cyclization of the side chains of monastrol leads to conformationally restricted bicyclic systems with enhanced inhibitory activity against Eg5. Enastron **2** (IC_{50} = 2 μM), enastrol **4** (IC_{50} = 2 μM), and dimethylenastron **3** (IC_{50} = 200 nM), were identified as potent and specific inhibitors (Table 2).[35] Furthermore, we discovered that the 3,4-dihydrophenylquinazoline-2(1*H*)-thione scaffold gives access to a new class of Eg5 inhibitors called Vasastrols (VS, Figure 2). The activity of VS-83 **5** was specific for Eg5 (IC_{50} = 1.2 μM) among nine kinesins tested, and this compound was the most potent inhibitor identified in this screening effort.[36]

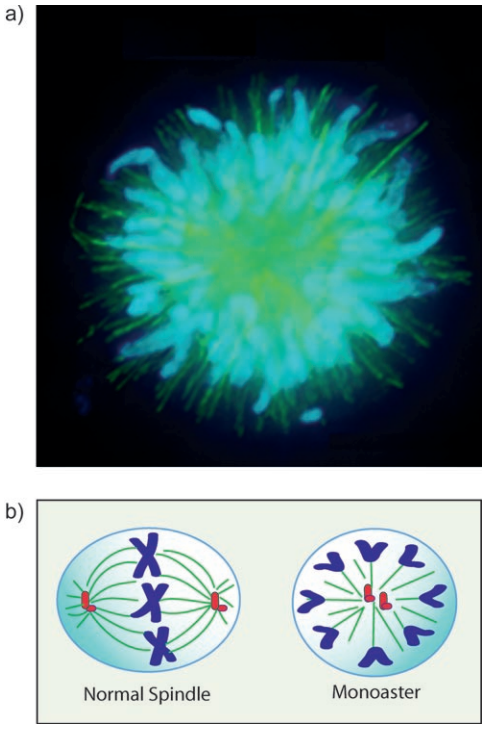


Figure 3. a) Representative confocal image of a monoaster after treatment with VS-83 (25 μM): cells were fixed and immunostained for the microtubule cytoskeleton and chromatin. b) Model of a normal bipolar spindle and a monoaster.

Table 2. Inhibitors of Eg5.

Compound	IC_{50} [nM]
(*S*)-monastrol **1**	14000
rac-enastron **2**	2000
rac-dimethylenastron **3**	200
rac-enastrol **4**	2000
rac-VS-83 **5**	1200
terpendole E (**6**)	23000
S-trityl-L-cysteine (**8**)	1000
HR22C16 (**9**)	800
trans-tetrahydro-β-carboline **10**	90
trans-tetrahydro-β-carboline **11**	650
dihydropyrazole **13**	26
KSP-IA (**14**)	11

Furthermore, the in vitro potencies of VS compounds are in good agreement with their efficiency to induce monoasters in BSC-1 cells.

Terpendole E (**6**) is the only natural product identified as an Eg5 inhibitor. It was isolated from a culture broth of a soil-isolated fungus and inhibits Eg5 ATPase activity with an IC_{50} value of 23 μM (Figure 4).[37] Another potent and allosteric inhibitor of Eg5 is the *R* enantiomer of CK0106023 (**7**, $K_i = 12$ nM for the racemic mixture), which exhibits antitumor activity in a variety of human tumor cell lines, including the three multidrug-resistant lines NCI/ADR-RES, HCT-15, and A2780ADR.[38] In tumor-bearing mice, **7** exhibited antitumor activity similar to that of

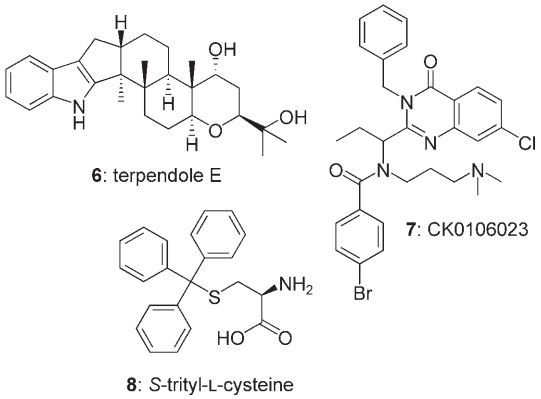


Figure 4. Eg5 inhibitors **6**–**8**.

paclitaxel, with a mean GI_{50} of 364 nM for the cell lines tested. Guided by **7**, a series of 2-(aminomethyl)quinazolinone derivatives have been identified that target Eg5. Among these, SB-715992 has antitumor activity in many murine and solid tumor models and is in phase II trials for evaluation in anticancer therapy.[39]

S-Trityl-L-cysteine (**8**), shown in Figure 4, was identified as an Eg5 inhibitor in an in vitro assay based screen of small-molecule libraries of a total of 2869 compounds obtained from the National Cancer Institute (NCI).[40] This amino acid derivative is an effective Eg5 inhibitor with an IC_{50} value of 1.0 μM in HeLa cells and an average GI_{50} value of 1.3 μM in NCI 60 tumor cell lines. Furthermore, despite their considerably different structures, both monastrol and *S*-trityl-L-cysteine bind to the same region in human Eg5, as was proven by H/D exchange mass spectrometry analysis in association with protein digestion and directed mutagenesis.[41]

The cell-division inhibitor HR22C16 (**9**), discovered from a forward chemical genetic screen of 16000 compounds, inhibits Eg5 with an IC_{50} value of 800 nM (Figure 5). The authors reported the solid-phase synthesis of a small library of 50 analogues of HR22C16[42] which were subsequently tested in cell-based assays. The most potent inhibitor was compound **10**, which inhibits Eg5 activity with an IC_{50} value of 90 nM. However, it has not been reported if this inhibitor is cell-permeable. A systematic investigation of the importance of stereochemistry and substituents in HR22C16 derivatives was performed by our research group. A small library of 60 tetrahydro-β-carbolines was synthesized and biologically evaluated.[43] These studies revealed that all four stereoisomers of HR22C16 are biologically active. Furthermore, the *trans*-tetrahydro-β-carboline **11** with an *N*-benzyl side chain as the R group proved to be a specific and potent inhibitor ($IC_{50} = 0.65$ μM, Figure 5).

Recently, dihydropyrazole **13** ($IC_{50} = 26$ nM) was reported to be an allosteric inhibitor of KSP as shown by X-ray crystallographic analysis (Figure 5). It induces apoptosis through the activation of caspase 3 in A270 human ovarian carcinoma cells.[44] By using a high-throughput screen, dihydropyrrole **14** ($IC_{50} =$ 11 nM) was also identified as a potent and specific KSP inhibitor that induces mitotic arrest and apoptosis in several human cancer cell lines (Figure 5).[45]

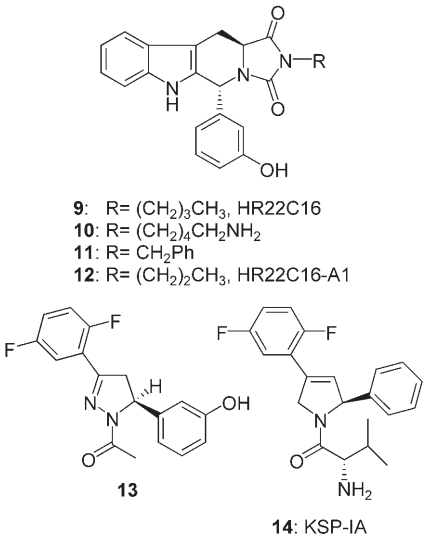


Figure 5. Eg5 inhibitors **9**–**14**.

Induction of Apoptosis

Previous studies have shown that Eg5 inhibitors induce mitotic arrest in PtK1 and PtK2 kidney cells of the kangaroo rat,[31] in BS-C-1 monkey epithelial kidney cells,[36] and in human HeLa cells.[37] The long-term effects of monastrol on cell proliferation were also examined in human AGS and HT29 cell lines from gastric and colon carcinomas. It was shown that monastrol causes mitotic arrest and induces early apoptosis through mitochondrial membrane depolarization, activation of caspases 8 and 3, and cleavage of poly-ADP-ribose polymerase 1 (PARP-1) with different sensitivities in the two cell lines.[46] Additionally, HR22C16 and its analogues induce cell death through the intrinsic apoptotic pathway in both taxol-sensitive A19 and taxol-resistant PTX10 and PTX22 human ovarian carcinoma cell lines, which have mutations in β-tubulin or those that cause overexpression of P-glycoprotein (PgP). HR22C16-A1 (**12**) induces antiproliferative activity and apoptotic cell death in PgP-overexpressing cells, indicating that **12** is not a PgP substrate (Figure 5). These data imply that Eg5 inhibition could be used as an alternative anticancer strategy to overcome taxane resistance in PgP-overexpressing cells.[47]

Like other drugs directed against microtubules, Eg5 inhibitors arrest cells in mitosis which generates signals for the activation of the spindle checkpoint. The mitotic spindle checkpoint monitors the microtubule attachments to chromosomes and delays chromosome segregation until the defects in the mitotic spindle are corrected.[48] If there is a lack of tension across sister kinetochores or if even a single kinetochore is not correctly attached to the spindle, the spindle checkpoint proteins Bub1, BubR1, Bub3, Mad1, and Mad2 are recruited and inhibit the anaphase-promoting complex/cyclosome (APC/C), which is responsible for the transition from metaphase to anaphase.[49]

Both tubulin-targeted drugs and Eg5 inhibitors cause sustained activation of the spindle checkpoint and therefore mitotic arrest, which often leads to the execution of cell death, but the complete mechanism still remains unclear. This is currently an important issue that needs to be explored for the clinical development of Eg5 inhibitors as chemotherapeutic agents.[50]

Recently, Tao et al. shed some light on the connections between mitotic arrest by a kinesin inhibitor and apoptosis.[51] They showed that apoptosis after long-term arrest, mediated by the pro-apoptotic protein Bax, cleavage of PARP, and activation of caspase 3 requires both activation of the spindle checkpoint and mitotic slippage in spindle-checkpoint-competent cells. Mitotic slippage is the process by which cells, in the presence of persistent spindle damage, exit mitosis to G1 without proper chromosome segregation and cytokinesis which results in tetraploid cells. Additionally, the authors suggest that the death resistance of HT29 cells, which sustain a long-term mitotic arrest after treatment with paclitaxel or KSP-IA (Figure 5), may be a result of delay in or resistance to mitotic slippage. Moreover, their findings support that the efficacy of KSP-IA could be enhanced by facilitating mitotic slippage with the Cdk1 inhibitor pulvalanol. This is the first insight into the mechanism of mitotic spindle checkpoint activation and apoptosis in response to anti-kinesin drugs, and further studies in this field are necessary.

Toxicity of the Inhibitors

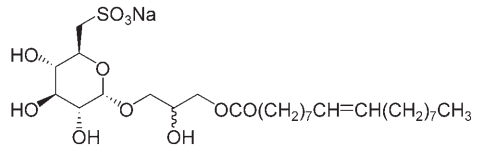
Neurons contain many cytoskeletal elements such as microtubules, actin filaments, and neurofilaments. They are terminally post-mitotic cells that use their microtubule arrays mostly for the formation of axons and dendrites. Interestingly, throughout the axon, microtubules are uniformly oriented, whereas in dendrites, the microtubules are non-uniformly oriented. It is now established that mitotic kinesins, which are responsible for the organization of the mitotic spindle and for the completion of mitosis, continue to be expressed in post-mitotic neurons well after mitotic division to regulate the microtubule arrays. For example, the kinesin CHO1/MKLP1 transports microtubules from the cell body into the dendrites and is necessary for the establishment of the non-uniform microtubule polarity for dendritic development.[52] Moreover, depletion of CHO1/MKLP1 from cultured neurons causes a rapid redistribution of microtubules within dendrites, changing their morphology and composition.[53] Another mitotic kinesin expressed in post-mitotic neurons is Eg5, which is implicated in microtubule regulation in the processes of newly developing neurons.[54] Additionally, Kif15 continues to be expressed in post-mitotic neurons and localizes to microtubules particularly in regions where oppositely oriented microtubules overlap; Kif15 opposes the forces of other kinesins in regions of developing processes.[55]

Recently, it was shown that the treatment of cultured sympathetic neurons with monastrol for a few hours enhances axonal growth, whereas longer exposure shows no toxicity, and neurons appear normal in terms of their morphology and microtubule organization. Sensory neurons seem to be more sensitive, and prolonged exposure results in shorter axons.[56] Moreover, the effect of monastrol in dendrites and axons was studied in primary cortical neuron cultures.[57] Monastrol-treat-

ed immature neurons had longer dendrites and shorter axons. In mature neurons, there were no significant differences between the control and monastrol-treated cells, but tubulin clustering was observed in axons. Therefore, further studies, especially with more potent inhibitors, are needed for the estimation of anti-kinesin drug toxicity in neurons. The roles of Eg5 in neurons is not completely clear, but the first results are promising. It seems that the toxic effects are modest compared with the deleterious effects of other antimitotics such as taxol.

MCAK Inhibition

Interestingly, the first MCAK inhibitors were recently reported by Aoki et al.[30] By using a phage-display method they discovered that the sulfoquinovosylacylglycerols (SQAGs, Figure 6), with C_{18} fatty acids on the glycerol moiety, arrest cells at the M phase and suppress microtubule polymerization by binding to the kinesin MCAK. Notably, small-molecule inhibitors of other mitotic kinesins, apart from Eg5 and MCAK, have not yet been reported. It is possible that the identification of such inhibitors could be difficult owing to functional redundancy between different mitotic kinesins.[15]



15: α-SQMG (18:1)

Figure 6. The α and β anomers of monoacyl and diacyl forms of SQAGs show inhibitory activity against MCAK.

Conclusions

Based on the available data discussed above, Eg5-targeted molecules could be used to overcome taxane resistance in taxol-resistant human cell lines. Although reports of PgP-mediated resistance for Eg5 inhibitors do not exist, this issue is a point of concern for further investigations. Furthermore, Eg5 antagonists display modest toxic effects in comparison with the toxicity of other antimitotics in neurons. The phase I results to determine the safety profile and pharmacokinetics of the 2-(aminomethyl)quinazolinone derivative SB-715992 in patients with multiple advanced solid tumors showed that this compound has an acceptable tolerance profile, and neurotoxicities were not observed.[58] The potential of mitotic kinesin inhibitors is unquestionable, but continued research and clinical evaluation are clearly necessary. The anticancer activity of these drug candidates may be augmented by their inhibitory action toward endothelial cell proliferation, resulting in the inhibition of angiogenesis. Finally, inhibitors of mitotic kinesins may find broad application for the treatment of other proliferative diseases such as diabetic retinopathy, restenosis, pulmonary and liver fibrosis, Sjögen's syndrome, lupus erythrematosus, and lymphoproliferative disorders that develop in patients with a history of autoimmune disease.

Keywords: antimitotics · antitumor agents · cell cycle · enzymes · inhibitors

[1] K. W. Wood, W. D. Cornwell, J. R. Jackson, *Curr. Opin. Pharmacol.* **2001**, *1*, 370 – 377.
[2] E. K. Rowinsky, *Annu. Rev. Med.* **1997**, *48*, 353 – 374.
[3] M. A. Jordan, L. Wilson, *Nat. Rev. Cancer* **2004**, *4*, 253 – 265.
[4] S. Quasthoff, H. P. Hartung, *J. Neurol.* **2002**, *249*, 9 – 17.
[5] M. M. Gottesman, T. Fojo, S. E. Bates, *Nat. Rev. Cancer* **2002**, *2*, 48 – 58.
[6] C. Dumontet, B. I. Sikic, *J. Clin. Oncol.* **1999**, *17*, 1061 – 1070.
[7] S. Sangrajrang, A. Fellous, *Chemotherapy* **2000**, *46*, 327 – 334.
[8] C. McInnes, M. Mezna, P. M. Fischer, *Curr. Top. Med. Chem.* **2005**, *5*, 181 – 197.
[9] N. Keen, S. Taylor, *Nat. Rev. Cancer* **2004**, *4*, 927 – 936.
[10] A. Yildiz, P. R. Selvin, *Trends Cell Biol.* **2005**, *15*, 112 – 120.
[11] N. Hirokawa, R. Takemura, *Exp. Cell Res.* **2004**, *301*, 50 – 59.
[12] H. Miki, M. Setou, K. Kaneshiro, N. Hirokawa, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 7004 – 7011.
[13] T. Wittmann, A. Hyman, A. Desai, *Nat. Cell Biol.* **2001**, *3*, E28 – 34.
[14] E. R. Hildebrandt, M. A. Hoyt, *Biochim. Biophys. Acta* **2000**, *1496*, 99 – 116.
[15] G. Goshima, R. D. Vale, *J. Cell Biol.* **2003**, *162*, 1003 – 1016.
[16] C. Zhu, J. Zhao, M. Bibikova, J. D. Leverson, E. Bossy-Wetzel, J.-B. Fan, R. T. Abraham, W. Jiang, *Mol. Biol. Cell* **2005**, *16*, 3187 – 3199.
[17] C. J. Lawrence, R. K. Dawe, K. R. Christie, D. W. Cleveland, S. C. Dawson, S. A. Endow, L. S. Goldstein, H. V. Goodson, N. Hirokawa, J. Howard, R. L. Malmberg, J. R. McIntosh, H. Miki, T. J. Mitchison, Y. Okada, A. S. N. Reddy, W. M. Saxton, M. Schliwa, J. M. Scholey, R. D. Vale, C. E. Walczak, L. Wordeman, *J. Cell Biol.* **2004**, *167*, 19 – 22.
[18] J. C. Cochran, C. A. Sontag, Z. Maliga, T. M. Kapoor, J. J. Correia, S. P. Gilbert, *J. Biol. Chem.* **2004**, *279*, 38861 – 38870.
[19] A. S. Kashina, R. J. Baskin, D. G. Cole, K. P. Wedman, W. M. Saxton, J. M. Scholey, *Nature* **1996**, *379*, 270 – 272.
[20] J. Turner, R. Anderson, J. Guo, C. Beraud, R. Fletterick, R. Sakowicz, *J. Biol. Chem.* **2001**, *276*, 25496 – 25502.
[21] T. M. Kapoor, T. J. Mitchison, *J. Cell Biol.* **2001**, *154*, 1125 – 1133.
[22] V. Mountain, C. Simerly, L. Howard, A. Ando, G. Schatten, D. A. Compton, *J. Cell Biol.* **1999**, *147*, 351 – 365.
[23] N. J. Ganem, D. A. Compton, *J. Cell Biol.* **2004**, *166*, 473 – 478.
[24] A. Moore, L. Wordeman, *Trends Cell Biol.* **2004**, *14*, 537 – 546.
[25] X. Yao, A. Abrieu, Y. Zheng, K. F. Sullivan, D. W. Cleveland, *Nat. Cell Biol.* **2000**, *2*, 484 – 491.
[26] K. Shiroguchi, M. Ohsugi, M. Edamatsu, T. Yamamoto, Y. Y. Toyoshima, *J. Biol. Chem.* **2003**, *278*, 22460 – 22465.
[27] C. Zhu, W. Jiang, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 343 – 348.
[28] C. Zhu, E. Bossy-Wetzel, W. Jiang, *Biochem. J.* **2005**, *389*, 373 – 381.
[29] U. Gruneberg, R. Neef, R. Honda, E. A. Nigg, F. A. Barr, *J. Cell Biol.* **2004**, *166*, 167 – 172.
[30] S. Aoki, K. Ohta, T. Yamazaki, F. Sugawara, K. Sakaguchi, *FEBS Lett.* **2005**, *272*, 2132 – 2140.
[31] T. U. Mayer, T. M. Kapoor, S. J. Haggarty, R. W. King, S. L. Schreiber, T. J. Mitchison, *Science* **1999**, *286*, 971 – 974.
[32] Z. Maliga, T. M. Kapoor, T. J. Mitchison, *Chem. Biol.* **2002**, *9*, 989 – 996.
[33] S. DeBonis, J. P. Simorre, I. Crevel, L. Lebeau, D. A. Skoufias, A. Blangy, C. Ebel, P. Gans, R. Cross, D. D. Hackney, R. H. Wade, F. Kozielski, *Biochemistry* **2003**, *42*, 338 – 349.
[34] Y. Yan, V. Sardana, B. Xu, C. Homnick, W. Halczenko, C. A. Buser, M. Schaber, G. D. Hartman, H. E. Huber, L. C. Kuo, *J. Mol. Biol.* **2004**, *335*, 547 – 554.
[35] M. Gartner, N. Sunder-Plassmann, J. Seiler, M. Utz, I. Vernos, T. Surrey, A. Giannis, *ChemBioChem* **2005**, *6*, 1173 – 1177.
[36] V. Sarli, S. Huemmer, N. Sunder-Plassmann, T. U. Mayer, A. Giannis, *ChemBioChem* **2005**, *6*, 2005 – 2013.
[37] J. Nakazawa, J. Yajima, T. Usui, M. Ueki, A. Takatsuki, M. Imoto, Y. Y. Toyoshima, H. Osada, *Chem. Biol.* **2003**, *10*, 131 – 137.

[38] R. Sakowicz, J. T. Finer, C. Beraud, A. Crompton, E. Lewis, A. Fritsch, Y. Lee, J. Mak, R. Moody, R. Turincio, J. C. Chabala, P. Gonzales, S. Roth, S. Weitman, K. W. Wood, *Cancer Res.* **2004**, *64*, 3276 – 3280.
[39] R. K. Johnson, F. L. McCabe, E. Cauder, L. Innlow, M. Whitacre, J. D. Winkler, G. Bergnes, B. Feng, W. W. Smith, D. Morgans, K. Wood, J. R. Jackson, *Proc. Am. Assoc. Cancer Res.* **2002**, *43*, 269.
[40] S. DeBonis, D. A. Skoufias, L. Lebeau, R. Lopez, G. Robin, R. L. Margolis, R. H. Wade, F. Kozielski, *Mol. Cancer Ther.* **2004**, *3*, 1079 – 1090.
[41] S. Brier, D. Lemaire, S. DeBonis, E. Forest, F. Kozielski, *Biochemistry* **2004**, *43*, 13 072 – 13 082.
[42] S. Hotha, J. C. Yarrow, J. G. Yang, S. Garrett, K. V. Renduchintala, T. U. Mayer, T. M. Kapoor, *Angew. Chem.* **2003**, *115*, 2481 – 2484; *Angew. Chem. Int. Ed.* **2003**, *42*, 2379 – 2382.
[43] N. Sunder-Plassmann, V. Sarli, M. Gartner, M. Utz, Z. Seiler, S. Huemmer, T. U. Mayer, T. Surrey, A. Giannis, *Bioorg. Med. Chem.* **2005**, *13*, 6094 – 6111.
[44] C. D. Cox, M. J. Breslin, B. J. Mariano, P. J. Coleman, C. A. Buser, E. S. Walsh, K. Hamilton, H. E. Huber, N. E. Kohl, M. Torrent, Y. Yan, L. C. Kuo, G. D. Hartman, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2041 – 2045.
[45] W. Tao, V. J. South, Y. Zhang, J. P. Davide, L. Farell, N. E. Kohl, L. Sepp-Lorenzino, R. B. Lobell, *Cancer Cell* **2005**, *8*, 49 – 59.
[46] I. Leizerman, R. Avunie-Masala, M. Elkabets, A. Fich, L. Gheber, *Cell. Mol. Life Sci.* **2004**, *61*, 2060 – 2070.
[47] A. I. Marcus, U. Peters, S. L. Thomas, S. Garrett, A. Zelnak, T. M. Kapoor, P. Giannakakou, *J. Biol. Chem.* **2005**, *280*, 11 569 – 11 577.
[48] A. Musacchio, K. G. Hardwick, *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 731 – 741.
[49] S. Kadura, S. Sazer, *Cell Motil. Cytoskeleton* **2005**, *61*, 145 – 160.
[50] B. A. A. Weaver, D. W. Cleveland, *Cancer Cell* **2005**, *8*, 7 – 12.
[51] W. Tao, V. J. South, Y. Zhang, J. P. Davide, L. Farrell, N. E. Kohl, L. Sepp-Lorenzino, R. B. Lobell, *Cancer Cell* **2005**, *8*, 49 – 59.
[52] D. J. Sharp, W. Yu, L. Ferhat, R. Kuriyama, D. C. Rueger, P. W. Baas, *J. Cell Biol.* **1997**, *138*, 833 – 843.
[53] W. Yu, C. Cook, C. Sauter, R. Kuriyama, P. L. Kaplan, P. W. Baas, *J. Neurosci.* **2000**, *20*, 5782 – 5791.
[54] L. Ferhat, C. Cook, M. Chauviere, M. Harper, M. Kress, G. E. Lyons, P. W. Baas, *J. Neurosci.* **1998**, *18*, 7822 – 7835.
[55] D. W. Buster, D. H. Baird, W. Yu, J. M. Solowska, M. Chauviere, A. Mazurek, M. Kress, P. W. Baas, *J. Neurocytol.* **2003**, *32*, 79 – 96.
[56] S. A. Haque, T. P. Hasaka, A. D. Brooks, P. V. Lobanov, P. W. Baas, *Cell Motil. Cytoskeleton* **2004**, *58*, 10 – 16.
[57] S. Y. Yoon, J. E. Choi, J.-W. Huh, O. Hwang, H. S. Lee, H. N. Hong, D. Kim, *Cell Motil. Cytoskeleton* **2005**, *60*, 181 – 190.
[58] http:www.clinicaltrials.gov

Received: September 10, 2005
Revised: September 30, 2005
Published online on January 12, 2006

