

Inhibitors of kinesin Eg5: antiproliferative activity of monastrol analogues against human glioblastoma cells

Christine Müller · Dietmar Gross · Vasiliki Sarli ·
Michael Gartner · Athanassios Giannis ·
Günther Bernhardt · Armin Buschauer

Received: 22 December 2005 / Accepted: 3 April 2006 / Published online: 16 May 2006
© Springer-Verlag 2006

Abstract The inhibition of kinesin Eg5 by small molecules such as monastrol is currently evaluated as an approach to develop a novel class of antiproliferative drugs for the treatment of malignant tumours. Therefore, we studied the effects of the new monastrol analogues enastron, dimethylenastron and vasastrol VS-83 on the proliferation of human glioblastoma cells in the kinetic crystal violet assay. Compared to monastrol, the new cell cycle specific compounds showed an at least one order of magnitude higher anti proliferative activity against U-87 MG, U-118 MG, and U-373 MG glioblastoma cells. The compounds were neither inactivated by hydrolysis nor by binding to serum proteins. Moreover, we demonstrated the characteristic monoaster formation after incubation of cells with the new compounds by confocal laser scanning microscopy. We also showed that the arrangement of β -actin and tubulin, vital components of the cytoskeleton of mitotic and quiescent cells, were not affected by the new compounds. Due to the necessity of overcoming the blood–brain barrier in the treatment of brain tumours, we investigated if the new monastrol analogues are modulators or substrates of the p-glycoprotein (p-gp) 170 by a flow cytometric calcein-AM efflux assay. The tested compounds showed no modulating effects on the p-gp function. With respect to the treatment of primary and secondary CNS tumours, the

results of our experiments suggest that the new monastrol analogues represent an interesting class of potential anticancer drugs, predicted to be less neurotoxic in comparison to classical tubulin inhibitors.

Keywords Kinesin inhibitors · Monastrol analogues · Human glioblastoma cells · Chemosensitivity · Confocal microscopy

Introduction

The inhibition of the mitotic kinesin Eg5 is an attractive new approach to cancer treatment [1]. Mitotic kinesins are exclusively involved in the formation and function of the mitotic spindle, and some kinesins are only expressed in proliferating cells. Eg5 mediates centrosome separation and the formation of the bipolar spindle. Inhibition of Eg5 leads to cell cycle arrest during mitosis, giving rise to cells with monopolar spindles, the so-called monoasters [2]. In contrast to classical mitotic inhibitors (vinca alkaloids and taxanes), Eg5 inhibitors do not interfere with other microtubule-dependent processes [1], which are the main reason for the neurotoxicity of anti-microtubule agents [3]. Racemic monastrol (Fig. 1), a 4-aryl-3,4-dihydro-pyrimidine-2(*IH*)-thione, was the first specific small-molecule inhibitor of Eg5 [4]. However, it is only a moderate allosteric inhibitor of Eg5 [5] with an IC_{50} value of 34 μ M, which has been determined in the microtubule-stimulated ATPase activity assay [6]. Therefore, new monastrol analogues were synthesized [7] (Fig. 1). We tested the antiproliferative effects of the derivatives enastron, dimethylenastron and vasastrol VS-83 on human glioblastoma/astrocytoma cells in

C. Müller · D. Gross · G. Bernhardt · A. Buschauer (✉)
Institute of Pharmacy, University of Regensburg,
Universitätsstraße 31, 93040 Regensburg, Germany
e-mail: armin.buschauer@chemie.uni-regensburg.de

V. Sarli · M. Gartner · A. Giannis
Institute of Organic Chemistry, University of Leipzig,
Johannisallee 29, 04103 Leipzig, Germany

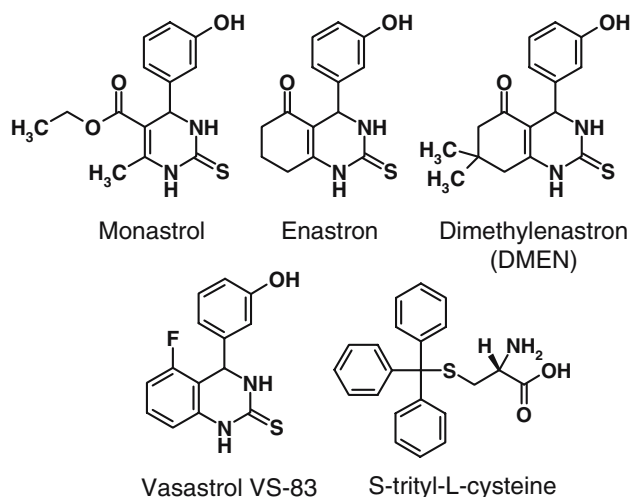


Fig. 1 Structures of monastrol, the unspecific kinesin inhibitor *S*-trityl-L-cysteine, and the new monastrol analogues enastron, dimethylenastron and vasastrol VS-83, utilized in chemosensitivity, microscopic and calcein-AM efflux studies

a chemosensitivity assay based on the crystal violet staining [8].

Furthermore, we investigated the effect of the new compounds on mitotic and quiescent human glioblastoma cells. The effects were compared to the effects of the antimicrotubular agent vinblastine, the selective kinesin Eg5 inhibitor monastrol and *S*-trityl-L-cysteine [9] (Fig. 1), which is also a specific kinesin inhibitor, though not selective for Eg5. The latter compounds served as controls for the characteristic monoaster formation during mitosis. The formation of the spindle apparatus as well as the arrangement of the cytoskeletal elements β -actin and tubulin, vital components of the cytoskeleton of mitotic and quiescent cells, were investigated by means of confocal laser scanning microscopy.

In the treatment of brain tumours, the Eg5 inhibitors have to overcome the blood–brain barrier. Therefore, we also investigated if the Eg5 inhibitors are modulators or substrates of the p-glycoprotein (p-gp) 170 using the calcein-AM efflux assay [10–12].

Materials and methods

Test compounds

The new monastrol analogues, enastron, dimethylenastron [5] and vasastrol VS-83 [7] (Fig. 1) were synthesized and characterized as described [5, 7]. *S*-trityl-L-cysteine [9] (Fig. 1) was from MP BIOMEDICALS, Eschwege, Germany, whereas monastrol was prepared

as described [5]. 10 mM stock solutions were prepared in DMSO; a 1 mM stock solution of vinblastine (SIGMA, München, Germany) was made in 70% ethanol. All compound stocks were stored at -20°C .

Cell lines and culture conditions

Human U-87 MG (HTB 14), U-118 MG (HTB 15), and U-373 MG (HTB 17) glioblastoma/astrocytoma cell lines were obtained from ATCC (Rockville, MD, USA). Cell banking and quality control were performed according to the “seed stock concept” [13]. U-87 MG and U-373 MG cells were grown in Eagle’s minimum essential medium containing L-glutamine, 2.2 g/l NaHCO_3 , 110 mg/l sodium pyruvate and 5% foetal calf serum (FCS), whereas the U-118 MG cells were maintained in Dulbecco’s minimal essential medium supplemented with 5% FCS. Kb-V1 cells, a p-gp overexpressing subclone [14] of Kb cells (ATCC CCL-17), were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% FCS and 300 ng/ml vinblastine. All cell types were maintained in water saturated atmosphere (95% air/5% carbon dioxide) at 37°C in 75-cm² culture flasks (NUNC, Wiesbaden, Germany) and were serially passaged following trypsinization using 0.05% trypsin/0.02% EDTA (ROCHE DIAGNOSTICS, Mannheim, Germany). Mycoplasma contamination was routinely monitored, and only Mycoplasma free cultures were used.

Chemosensitivity assay

The assays were performed as described previously [8]. In brief, tumour cell suspensions (100 μl /well) were seeded into 96-well flat-bottomed microtitration plates (GREINER, Frickenhausen, Germany) at a density of ca. 15 cells/microscopic field (magnification 320-fold). After 2–3 days, the culture medium was removed by suction and replaced by fresh medium (200 μl /well) containing varying drug concentrations or vehicle. Drugs were added as 1,000-fold concentrated feed solutions. On every plate 16 wells served as controls and 16 wells were used per drug concentration. After various times of incubation the cells were fixed with glutardialdehyde (Merck, Darmstadt, Germany) and stored in a refrigerator. At the end of the experiment, all plates were processed simultaneously [staining with 0.02% aqueous crystal violet (SERVA, Heidelberg, Germany) solution (100 μl /well)]. Excess dye was removed by rinsing the trays with water for 20 min. The stain bound by the cells was redissolved in 70% ethanol (180 μl /well) while shaking the microplates for about

3 h. Absorbance (a parameter proportional to cell mass) was measured at 578 nm using a BIOTEK 309 Autoreader (TECNOMARA, Fernwald, Germany).

Drug effects were expressed as corrected T/C -values for each group according to

$$T/C = (T - C_0)/(C - C_0) \times 100 \quad (\%) \quad (1)$$

where T is the mean absorbance of the treated cells, C the mean absorbance of the controls and C_0 the mean absorbance of the cells at the time ($t = 0$) when drug was added.

Confocal laser scanning microscopy

Treatment of the cells

Cells were seeded into 8-well Lab-Tek Chamber Slides (NUNC, Wiesbaden, Germany). At 75% confluence, the culture medium was replaced with medium containing 50 μ M monastrol, 1 μ M *S*-trityl-L-cysteine or 10 nM vinblastine. The concentrations of the new monastrol analogues were selected on the basis of chemosensitivity data, obtained by the kinetic crystal violet assay. Cells were incubated at 37°C for 2 h.

Fixation and permeabilization of the glioblastoma cells

After the incubation with drugs, the medium was carefully removed, and the cells were fixed with 4% paraformaldehyde solution in phosphate buffered saline (PBS) for 20 min at room temperature. Thereafter, each well was washed three times with PBS containing 0.5% bovine serum albumin (BSA, SERVA, Heidelberg, Germany). Cells were permeabilized by incubation with PBS containing 0.5% BSA and 1% Triton-X 100 (SERVA, Heidelberg, Germany) for 10 min at room temperature, followed by three washing steps with PBS 0.5% BSA.

Fluorescent staining and immunofluorescence

Chromosomes were stained with SYTOXGreen® nucleic acid staining dye (MOLECULAR PROBES, Eugene, OR, USA). Microtubules were stained using mouse anti-human α -tubulin primary antibody (DIANOVA, Hamburg, Germany) and Alexa Fluor®546 conjugated goat anti-mouse secondary antibody (MOLECULAR PROBES). All antibodies were used in a 1:200 dilution in PBS, containing 0.5% BSA. β -Actin was stained with Alexa Fluor®647 phalloidin (MOLECULAR PROBES, Eugene, OR, USA).

Microscopic images were acquired with a Carl Zeiss Axiovert 200M LSM510 confocal laser scanning microscope. Multifluorescence image acquisition was performed in multitrack acquisition mode using the following parameters: SYTOXGreen®, 488 nm Argon laser, 505 nm longpass filter, Alexa Fluor® 546, 543 nm HeNe laser, 560–615 nm bandpass filter, Alexa Fluor® 647, 633 nm HeNe laser, 650 nm longpass filter.

Image processing

False colours were used for β -actin (red), tubulin (green) and DNA (blue). Images (Fig. 5a) were processed with the AutoDeBlur® deconvolution software.

Flow cytometric measurement of calcein-AM efflux

In Kb-V1 cells, calcein-AM is extruded by p-gp before esterases can cleave the ester bonds, and calcein is not accumulated [11]. Therefore, modulators of the p-gp function can easily be recognized by flow cytometric measurement of the change in the calcein-AM efflux.

Calcein-AM (MOLECULAR PROBES Eugene, OR, USA) was dissolved in DMSO (MERCK, Darmstadt, Germany) to achieve a final concentration of 1 mM and aliquoted into stock solutions that were stored at -20°C . Elacridar, a 3rd generation p-gp inhibitor (kindly provided by GlaxoSmithKline, NC, USA) served as a positive control for the inhibition of calcein-AM efflux.

Kb-V1 cells were trypsinized 3 or 4 days after the passaging and washed with PBS at 25°C. To 0.75 ml cell suspension containing 1×10^6 cells in loading buffer (120 mM NaCl, 5 mM KCl, 2 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.5 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 25 mM HEPES, 10 mM glucose, pH 7.4) 0.25 ml loading suspension (loading buffer, 20 mg/ml BSA, 5 μ l/ml of Pluronic® F127 [MOLECULAR PROBES, Eugene, OR, USA, 20% stock DMSO]) was added. The samples were mixed with different concentrations of test compounds and vortexed. After 15 min, calcein-AM solution was added to achieve a concentration of 1 μ M. After incubation for 10 min at 37°C/5% CO_2 the supernatant was discarded after centrifugation for 7 min at 4°C and 1,100 rpm. The cell pellet was rinsed once with ice cold PBS and resuspended in 0.5 ml of loading buffer per 1×10^6 cells. Calcein fluorescence was measured by a FACS Calibur™ (BECTON DICKINSON, Heidelberg, Germany) flow cytometer in the FL1-H channel. In each measurement 30,000 gated events were evaluated. The photomultiplier settings were as follows: E-1 for FSC, 270 for SSC and 300

for FL1-H. Data were analysed by the WinMDI 2.8 software.

Results

Drug exposure and chemosensitivity

Glioblastoma cells were treated with monastrol, enastron, dimethylenastron and vasastrol VS-83 at various concentrations up to 10 μM 48 h after seeding. In these experiments, the drug (and vehicle) containing culture media were not exchanged during the whole incubation period. The results are exemplarily summarized for U-373 MG cells in Fig. 2. In these plots of T/C_{corr} versus time of incubation $t = 0$ indicates the time when drug was added. According to Eq. 1, any growth curve of a drug treated cell population can be reconstructed

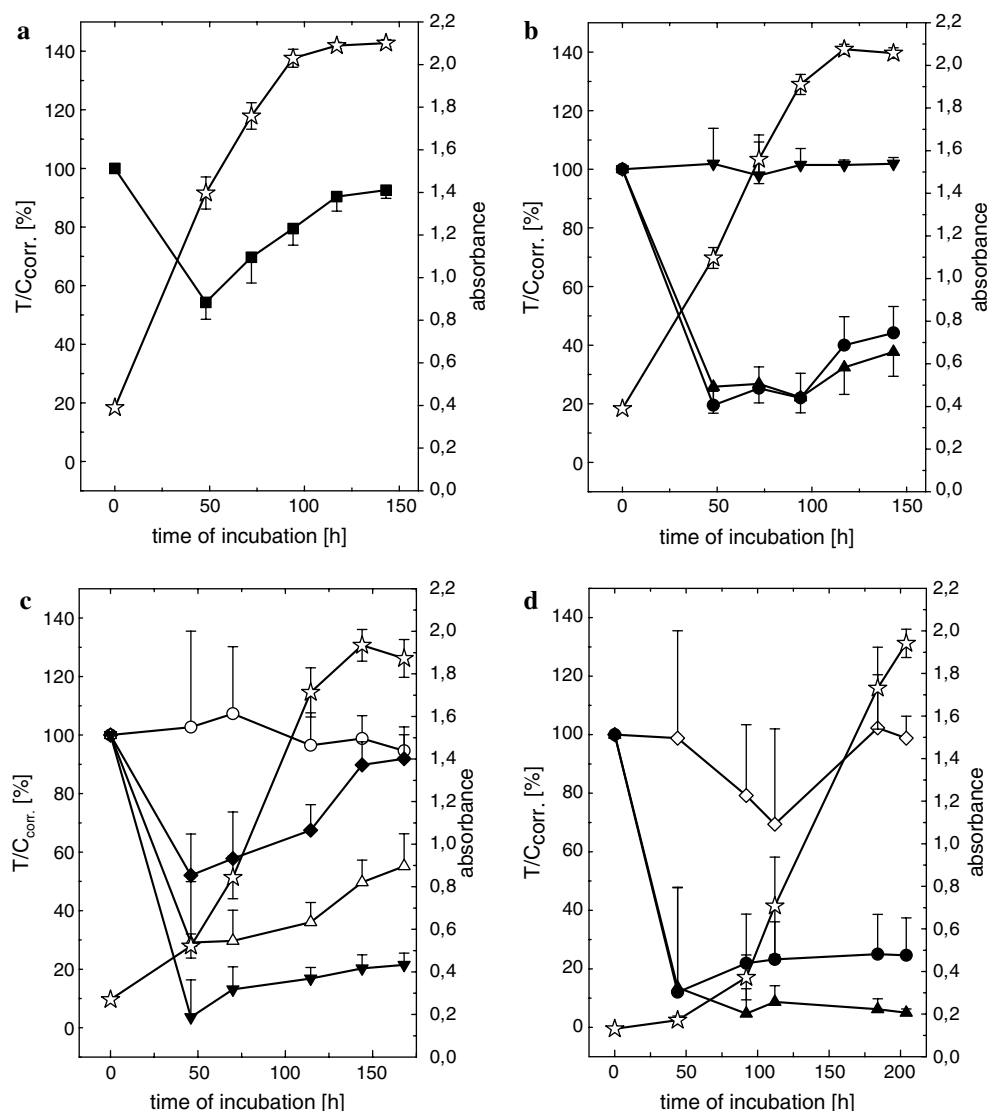
from the T/C_{corr} values and the data from the growth curve of the corresponding control [8].

For enastron and the vasastrol VS-83 a cytotoxic effect was observed at a concentration of 5 μM (Fig. 2b, d). A cytostatic effect (0% T/C_{corr} towards the end of the incubation period, i.e., 100% inhibition of net cell proliferation) was observed at 1 μM of dimethylenastron (Fig. 2c). Compared to monastrol (Fig. 2a), the three new compounds showed an at least one order of magnitude higher antiproliferative activity against the glioblastoma cells.

Pre-incubation of the cells with new monastrol analogues

To consider potential inactivation of the compounds by esterases or binding to serum proteins, we pre-incubated monastrol (50 μM) and dimethylenastron (1 μM)

Fig. 2 Proliferation kinetics of U-373 MG cells (passage 317) during permanent incubation with monastrol (a), the new monastrol analogues enastron (b), dimethylenastron (c) and vasastrol VS-83 (d). Open star vehicle, open circle 0.25 μM , filled diamond 0.5 μM , open triangle 0.75 μM , filled inverted triangle 1 μM , open diamond 3 μM , filled circle 5 μM , filled triangle 10 μM and filled square 50 μM



in culture medium for 1, 3 (data not shown) and 6 h. Then, the cells (U-87 MG, U-118 MG, and U-373 MG) were continuously incubated with culture medium containing the pre-incubated compounds. As shown for U-373 MG cells in Fig. 3, the antiproliferative effects of monastrol and dimethylenastron were retained compared to permanent drug exposure of the cells (Fig. 2a, c), indicating that the compounds were neither inactivated by hydrolysis nor by binding to serum proteins.

Effect of new monastrol analogues on quiescent cells

Confluent cells were treated with Eg5 inhibitors to compare their effects with those of anti-tubulin drugs like paclitaxel against resting cells. Rotenone, an ubiquinon reductase inhibitor, blocking ATP synthesis, served as a positive control for maximum cytotoxic effect. The results are shown in Fig. 4. Cell death was observed as expected for the rotenone treated cells. In contrast to paclitaxel, all tested Eg5 inhibitors had no effect on resting cells. All results described above were qualitatively and quantitatively similar for human U-87 MG and U-118 MG glioblastoma/astrocytoma cells (results not shown).

Effect of the new monastrol analogues on the spindle apparatus

According to the results of the chemosensitivity tests, human U-87 MG cells were incubated with 5 μ M enastron, 1 μ M dimethylenastron and 5 μ M vasastrol VS-83 for 2 h. As shown in Fig. 5a, the compounds enastron and dimethylenastron produced the same characteristic effect on the formation of the mitotic

spindle as the selective Eg5 kinesin inhibitor monastrol. However, the new compounds induced monoasters at lower concentrations, confirming the observations of monoaster formation in other cell types [5]. A different monoaster phenotype was observed after incubation with VS-83, similar to monoaster formation induced by the non-selective kinesin inhibitor S-trityl-L-cysteine (Fig. 5a).

Effect of the new monastrol analogues on the cytoskeleton of resting cells

While vinblastine treatment resulted in the disruption of the tubulin assembly in resting cells, the new monastrol analogues showed no effect on microtubular structures (Fig. 5b). Neither was the β -actin cytoskeleton affected by the new compounds, exemplarily shown for dimethylenastron (Fig. 5d). No differences to the morphology of the untreated cells (Fig. 5c) were observed.

Calcein-AM efflux assay

We used the 3rd generation p-gp inhibitor elacridar [15] as positive control. The new kinesin Eg5 inhibitors dimethylenastron (Fig. 6) and VS-83 did not inhibit p-gp activity up to a concentration of 20 μ M.

Discussion

In the present study we demonstrated that, compared to the first selective small molecule Eg5 kinesin inhibitor monastrol, the newly synthesized monastrol analogues enastron, dimethylenastron and vasastrol VS-83 exhibit higher antiproliferative activity against

Fig. 3 Incubation of U-373 MG cells after pre-incubation of 50 μ M monastrol (a) and 1 μ M dimethylenastron (b) in culture medium supplemented with 5% FCS. Open star vehicle, filled square 1 h and filled triangle 6 h pre-incubation period

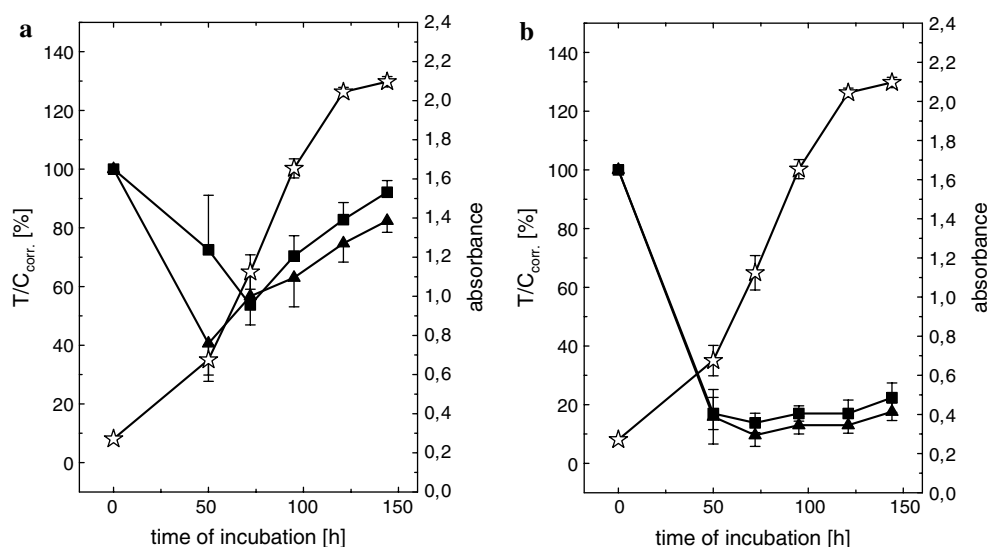


Fig. 4 Incubation of resting U-373 MG **(a)** and U-87 MG cells **(b)** with paclitaxel, rotenone and new monastrol analogues. *Open star* vehicle, *filled square* paclitaxel, *filled triangle* rotenone, *filled diamond* dimethylenastron and *filled circle* enastron

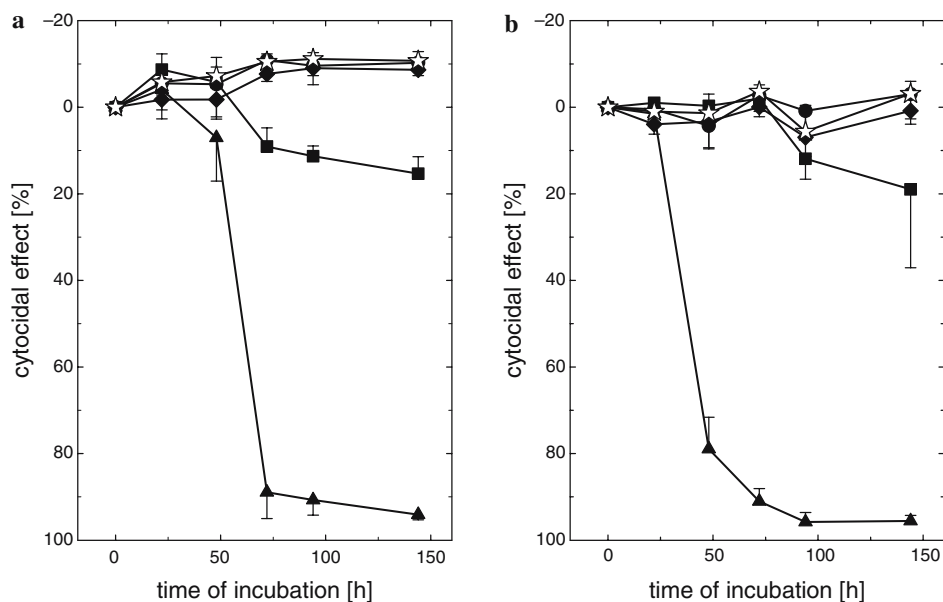
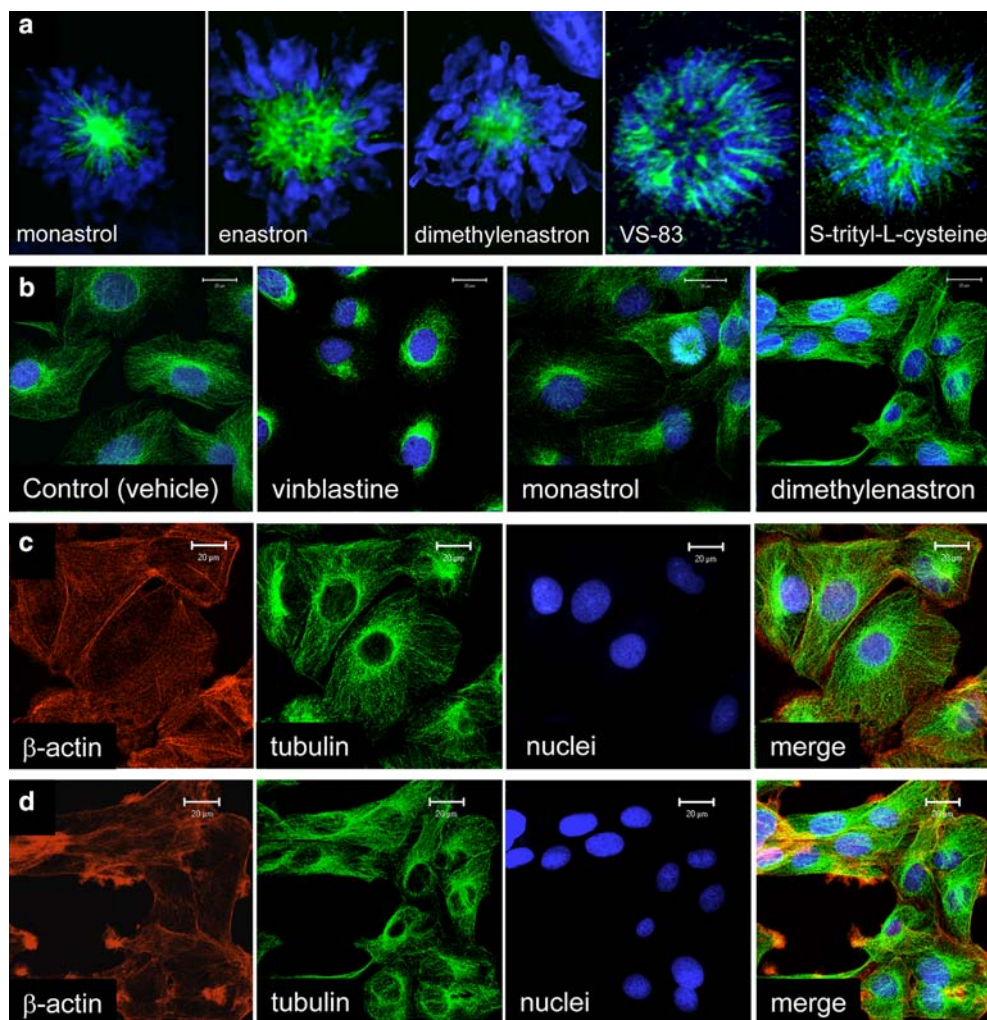


Fig. 5 a Formation of monoastrol spindles in human U-87 MG cells after incubation with monastrol (50 μ M), the new monastrol analogues enastron (1 μ M), dimethylenastron (1 μ M), vasastrol VS-83 (5 μ M), and S-trityl-L-cysteine (1 μ M); merged images of stained spindle and chromosomes. **b** Human U-87 MG glioblastoma cells, incubation with 10 nM vinblastine, 1 μ M S-trityl-L-cysteine and dimethylenastron (1 μ M). **c** Resting U-87 MG cells after incubation with 1 μ M dimethylenastron and **d** untreated cells. In quiescent cells, the microtubular and the β -actin cytoskeleton are not affected by the kinesin inhibiting compound



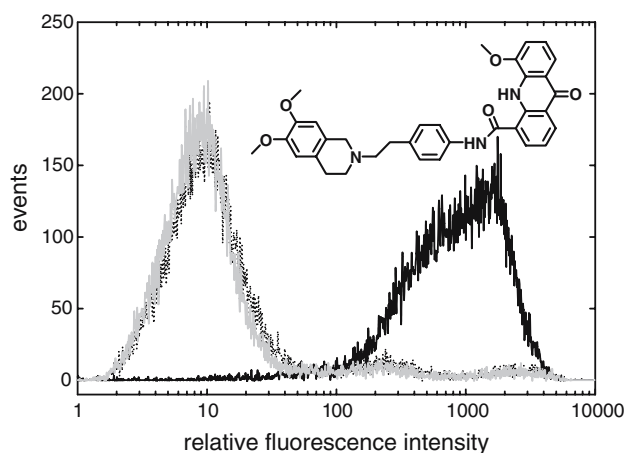


Fig. 6 Calcein-AM efflux from KbV1 cells after incubation with vehicle (dotted), 50 nM elacridar (black) and 20 μ M dimethylenastron (grey). Overlay histograms of gated Kb-V1 cells using fluorescence 1 (BP 530/30 nm). Structural formula: elacridar

human glioblastoma cells. Enastron and vasastrol VS-83 showed cytotoxic effects at a concentration of 5 μ M whereas monastrol was less effective even at 50 μ M. For dimethylenastron, an even higher antiproliferative potency was observed. The latter monastrol analogue was identified as the most potent compound with an approximately 100-fold higher antiproliferative efficacy compared to monastrol.

The antiproliferative activity of all tested monastrol derivatives was not reduced by the pre-incubation in serum supplemented culture medium, indicating that the compounds were not inactivated by esterase cleavage or by binding to serum proteins.

By analogy to the results of the chemosensitivity experiments, our microscopic studies showed that the new monastrol analogues give rise to mitotic arrest in human glioblastoma cells by inducing the formation of monoasters, the characteristic effect of the Eg5 kinesin inhibition. This effect was observed after 2 h of drug exposure. In contrast to the work of Sarli et al. [7], reporting on similar effects of monastrol and VS-83, in our studies the incubation with VS-83 led to a different monoaster phenotype, which was also observed after incubation with *S*-trityl-L-cysteine. However, Sarli et al. [7] applied 100 μ M monastrol and 25 μ M VS-83 to synchronized simian BSC-1 cells. In addition, the BSC-1 cells were incubated for 10 h, whereas the human U-87 MG glioblastoma cells were treated with monastrol (50 μ M) and VS-83 (5 μ M) for 2 h.

In contrast to the antimicrotubular agent paclitaxel, none of the tested Eg5 inhibitors had a cytotoxic effect on resting cells. These results were supported by microscopic investigations on the effect of the new monastrol analogues on the microtubules and the

β -actin (cytoskeleton) of resting cells. Thus, these compounds are supposed to be less neurotoxic anticancer drugs in comparison to classical tubulin inhibitors.

Since the p-gp efflux pump, located at the blood-brain barrier, is suggested to be the major reason for multidrug resistance of brain tumours against numerous anticancer agents [16], we investigated the effect of the new Eg5 kinesin inhibitors on the p-gp function using the flow cytometric calcein-AM efflux assay. While the 3rd generation p-gp inhibitor elacridar showed a high p-gp inhibition and consequently a strong calcein accumulation in Kb-V1 cells, no difference between the vehicle control and the tested Eg5 inhibitors was observed. This demonstrates that the new compounds are neither modulators nor substrates of the p-gp 170 up to 20 μ M, a concentration much higher than required for monoaster formation and the inhibition of cell proliferation, respectively. From a therapeutic point of view, p-gp inhibition at higher concentrations (f2 value of 113 μ M), as recently described for monastrol [17], seems to be of minor relevance.

In summary our results suggest that the new monastrol analogues enastron and its derivative dimethylenastron as well as the vasastrol VS-83 represent an interesting class of potential anticancer drugs, whereas dimethylenastron (ATPase IC_{50} 200 nM) represents a promising lead structure for the development of further new derivatives with even higher potency for the inhibition of the Eg5 kinesin. This is supported by the report of Tarby et al. [18], who developed a potent Eg5 inhibitor, derived from the 4-phenyl-tetrahydroisoquinoline lead series. The compound showed an IC_{50} value of 104 nM in an ATPase assay and induced the characteristic monoastralic spindles in PtK2 cells.

Enastron, dimethylenastron and VS-83 were ineffective against resting cells so that these compounds should be less neurotoxic in comparison to classical tubulin inhibitors. As the monastrol analogues are neither substrates nor modulators of p-gp 170, these Eg5 inhibitors may be promising new agents for further preclinical studies aiming at the treatment of primary and secondary CNS tumours.

Acknowledgements This work was supported by Grant MRTN CT-2004-512348 (Spindle Dynamics) from the European Commission. We also thank the Fonds der Chemischen Industrie for financial support.

References

1. Wood KW, Cornwell WD, Jackson JR (2001) Past and future of the mitotic spindle as an oncology target. *Curr Opin Pharmacol* 1:370–377

2. Blangy A, Lane HA, dHerin P, Harper M, Kress M, Nigg EA (1995) Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. *Cell* 83:1159–1169
3. Quasthoff S, Hartung HP (2002) Chemotherapy-induced peripheral neuropathy. *J Neurol* 249:9–17
4. Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ (1999) Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. *Science* 286:971–974
5. Gartner M, Sunder-Plassmann N, Seiler J, Utz M, Vernos I, Surrey T, Giannis A (2005) Development and biological evaluation of potent and specific inhibitors of mitotic Kinesin Eg5. *Chembiochem* 6:1173–1177
6. Maliga Z, Kapoor TM, Mitchison TJ (2002) Evidence that monastrol is an allosteric inhibitor of the mitotic kinesin Eg5. *Chem Biol* 9:989–996
7. Sarli V, Huemmer S, Sunder-Plassmann N, Mayer TU, Giannis A (2005) Synthesis and biological evaluation of novel Eg5 inhibitors. *Chembiochem* 6:2005–2013
8. Bernhardt G, Reile H, Birnbock H, Spruss T, Schonenberger H (1992) Standardized kinetic microassay to quantify differential chemosensitivity on the basis of proliferative activity. *J Cancer Res Clin Oncol* 118:35–43
9. Brier S, Lemaire D, Debonis S, Forest E, Kozielski F (2004) Identification of the protein binding region of *S*-trityl-L-cysteine, a new potent inhibitor of the mitotic kinesin Eg5. *Biochemistry* 43:13072–13082
10. Hollo Z, Homolya L, Davis CW, Sarkadi B (1994) Calcein accumulation as a fluorometric functional assay of the multidrug transporter. *Biochim Biophys Acta* 1191:384–388
11. Homolya L, Hollo Z, Germann UA, Pastan I, Gottesman MM, Sarkadi B (1993) Fluorescent cellular indicators are extruded by the multidrug resistance protein. *J Biol Chem* 268:21493–21496
12. Homolya L, Hollo M, Muller M, Mechetner EB, Sarkadi B (1996) A new method for a quantitative assessment of P-glycoprotein-related multidrug resistance in tumour cells. *Br J Cancer* 73:849–855
13. Hay RJ (1988) The seed stock concept and quality control for cell lines, *Anal Biochem* 171:225–237
14. Hubensack M (2005) Approaches to overcome the blood-brain barrier in the chemotherapy of primary and secondary brain tumors: modulation of P-glycoprotein 170 and targeting of the transferrin receptor. Doctoral thesis, University of Regensburg, <http://www.opus-bayern.de/uni-regensburg/volltexte/2005/471/>
15. Kemper EM, van Zandbergen AE, Cleypool C, Mos HA, Boogerd W, Beijnen JH, van Tellingen O (2003) Increased penetration of paclitaxel into the brain by inhibition of P-glycoprotein. *Clin Cancer Res* 9:2849–2855
16. Fellner S, Bauer B, Miller DS, Schaffrik M, Fankhänel M, Spruß T, Bernhardt G, Graeff C, Färber L, Gschaidmeier H, Buschauer A, Fricker G (2002) Transport of paclitaxel (Taxol) across the blood-brain barrier in vitro and in vivo. *J Clin Invest* 110:1309–1318
17. Peters T, Lindenmaier H, Haefeli WE, Weiss J (2006) Interaction of the mitotic kinesin Eg5 inhibitor monastrol with P-glycoprotein. *Naunyn Schmiedebergs Arch Pharmacol* 372:291–299
18. Tarby CM, Kaltenbach RF, III, Huynh T, Pudzianowski A, Shen H, Ortega-Nanos M, Sheriff S, Newitt JA, McDonnell PA, Burford N, Fairchild CR, Vaccaro W, Chen Z, Borzilleri RM, Naglich J, Lombardo LJ, Gottardis M, Trainor GL, Roussel DL (2006) Inhibitors of human mitotic kinesin Eg5: characterization of the 4-phenyl-tetrahydroisoquinoline lead series. *Bioorg Med Chem Lett* 16:2095–2100