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Investigation of pharmacokinetic properties of CK2 Inhibitors with an Indeno[1,2-*b*]indole scaffold

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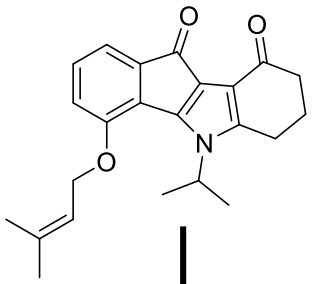
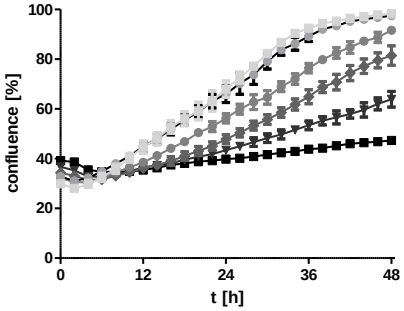
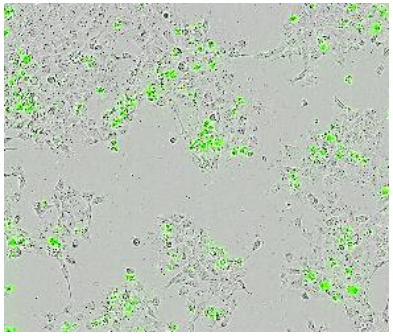
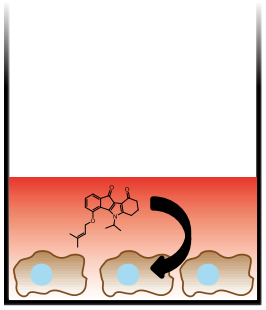
Investigation of pharmacokinetic properties of CK2 Inhibitors with an Indeno[1,2-*b*]indole scaffold

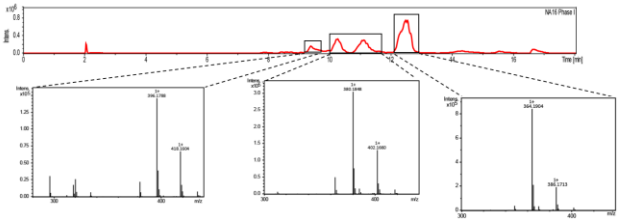
Graphical Abstract











Influence on tumor cell growth

Induction of apoptosis

Uptake analysis

Metabolism studies

Abstract:

The highly pleiotropic and constitutively active protein kinase CK2 plays an important role in several cellular mechanisms. Due to its overexpression and elevated activity in tumor cells, CK2 became an important target in tumor therapy nowadays. It was shown, that the kinase causes antiapoptotic and proliferation enhancing effects in neoplastic tissues [1,2]. Moreover, the reduction of CK2 activity in tumor cells leads to apoptosis while normal cells stay unaffected [3].

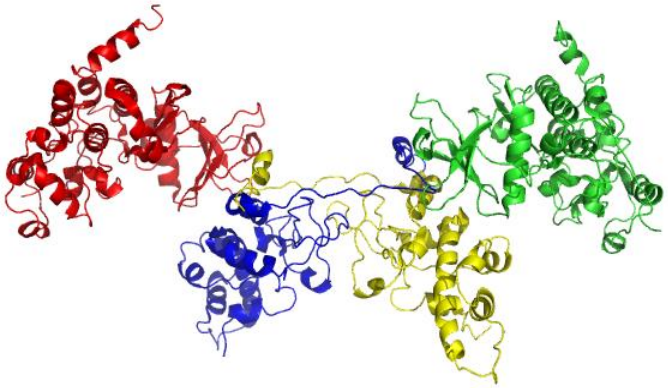
Indeno[1,2-*b*]indoles are ATP competitive CK2 inhibitors with IC_{50} values in the nanomolar range of concentration. NA16 was described as one of the most potent indeno[1,2-*b*]indoles with an IC_{50} value of 25 nM [4]. Therefore, the pharmacokinetic properties of NA16 were further analyzed during this study. It could be shown that NA16 reduces the growth of different tumor cell lines and induces cancer cell apoptosis. Furthermore, its effect on HUVEC cell growth was comparable with the effect of CX-4945, a CK2 inhibitor in clinical trials [5], which was used as control. Intracellular concentrations of NA16 were higher than concentrations of CX-4945 at different time points. Metabolism studies showed that NA16 is moderate metabolic stable and is not glucuronidated. These results underline the potential of NA16 as an antitumor drug.

References:
[1] Ahmed, K *et al.*: *Trends Cell Biol* **2002, 12, 226-230.**
[2] Meggio, F. and Pinna, L.A.: *FASEB J.* **2003, 17, 349-368.**
[3] Slaton, J. W. *et al.*: *Mol Cancer Res* **2004, 2, 172.**
[4] Gozzi, G. J. *et al.*: *J Med Chem* **2015, 58, 265-277.**
[5] Siddiqui-Jain, A. *et al.*: *Cancer Res* **2010, 70, 24.**

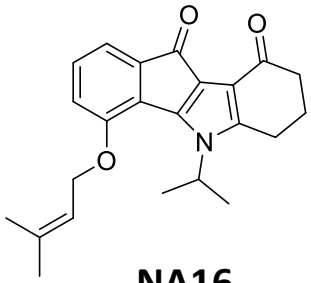
Keywords: Protein kinase CK2; ATP-competitive inhibitor; cell culture; pharmacokinetic

Introduction

- CK2:
  - Heterotetrameric holoenzyme
  - Ubiquitous Ser/Thr kinase
  - Constitutively active; highly pleiotropic
  - **Overexpression and higher activity in tumor cells**
  - **Important target in tumor therapy**

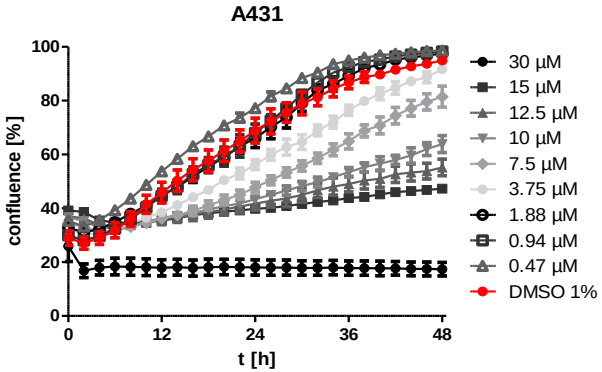


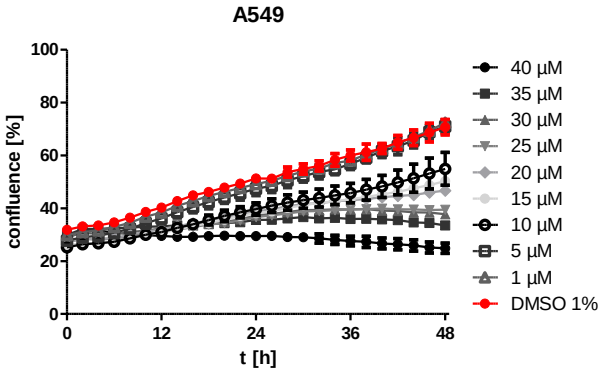
- Indeno[1,2-*b*]indoles:
  - Potent, ATP competitive CK2 inhibitors
  - Low IC_{50} values: <1 µM
  - **NA16: One of the most potent Indeno[1,2-*b*]indole derivatives**
    - **IC_{50} = 25 nM**

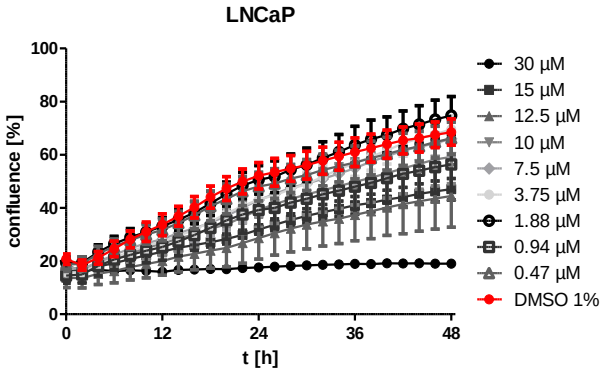


NA16

Results and discussion
1. Influence of NA16 on tumor cell growth







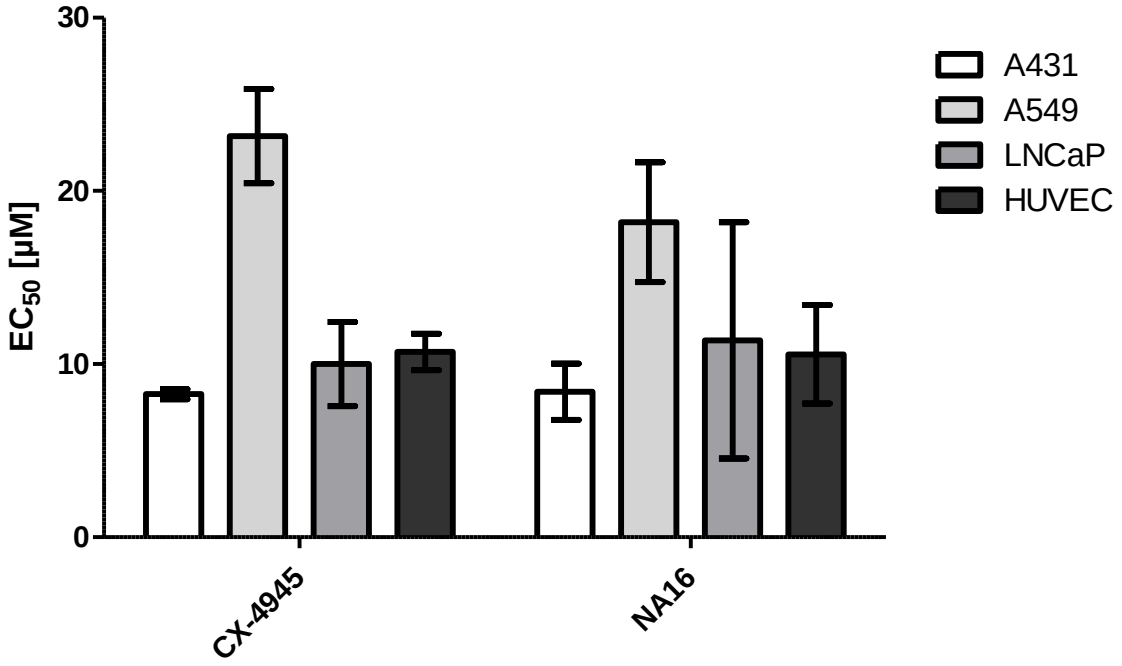
- Investigation of tumor cell growth via Live Cell Imaging
- Analyzing the effect of NA16 on growth of different cancer cell lines: A431 (epidermal), A549 (lung) and LNCaP (prostate)

- **NA16 reduces growth of all tested tumor cell line dose-dependently**

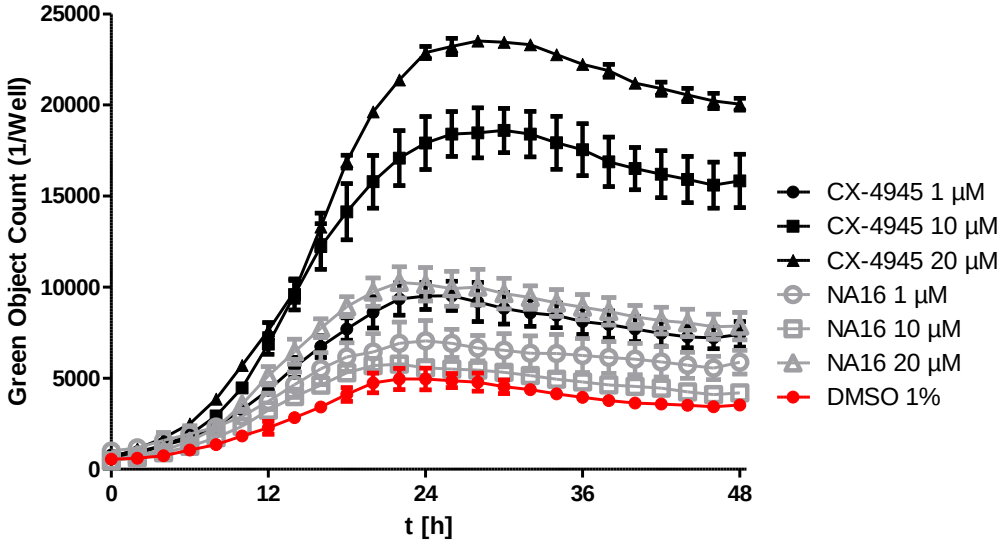
Results and discussion
1. Influence of NA16 on tumor and normal cell growth

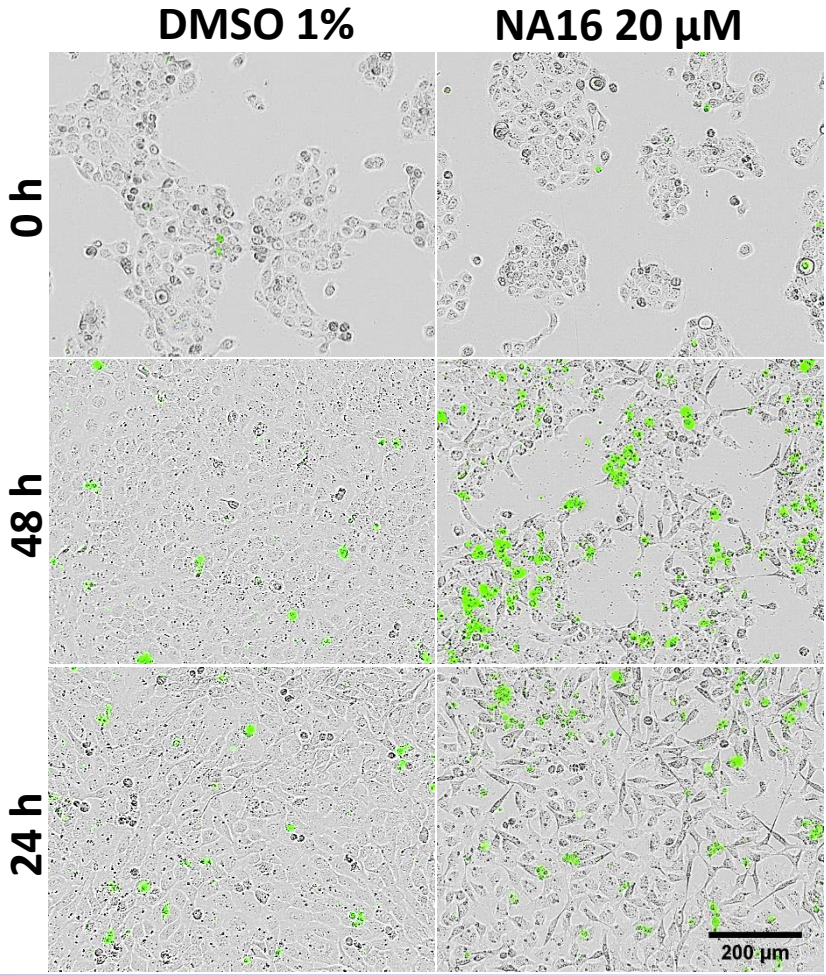
- EC_{50}: Inhibitor concentration which causes a reduction of cell growth by 50% compared to cells treated with 1% DMSO
- HUVEC: Human umbilical vein endothelial cell
  - ➢ Non-cancer cell line

- NA16 reduces tumor cell growth in the same dimension as CX-4945
- EC_{50} of NA16 on HUVEC cells comparable to EC_{50} of CX-4945



Results and discussion
2. Induction of apoptosis by NA16



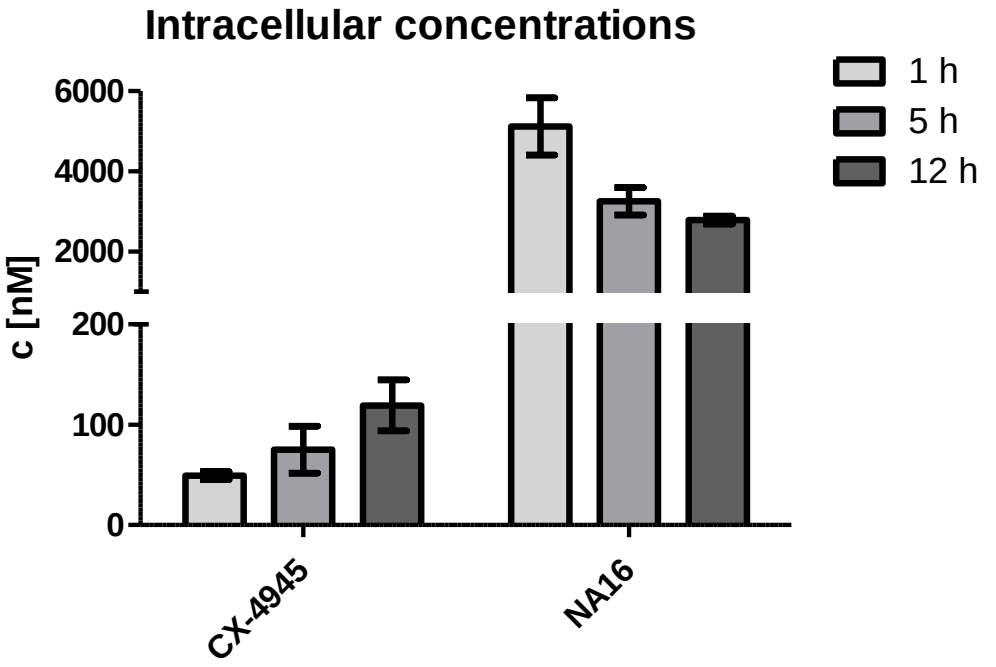


- Apoptosis analysis of A431 cells via Live Cell Imaging
- Fluorescence labeling of apoptotic cells after treatment with CK2 inhibitors
  - ➢ Induction of apoptosis detectable as well after treatment with CX-4945 as with NA16

Results and discussion
3. Cellular uptake

- Uptake analysis of CK2 inhibitors into A431 cells
- Extracellular concentration: 1 µM
- Different incubation times: 1 h, 5 h, 12 h
- Cell lysis and sample purification
- Quantification of intracellular inhibitor concentration via HPLC-MS/MS

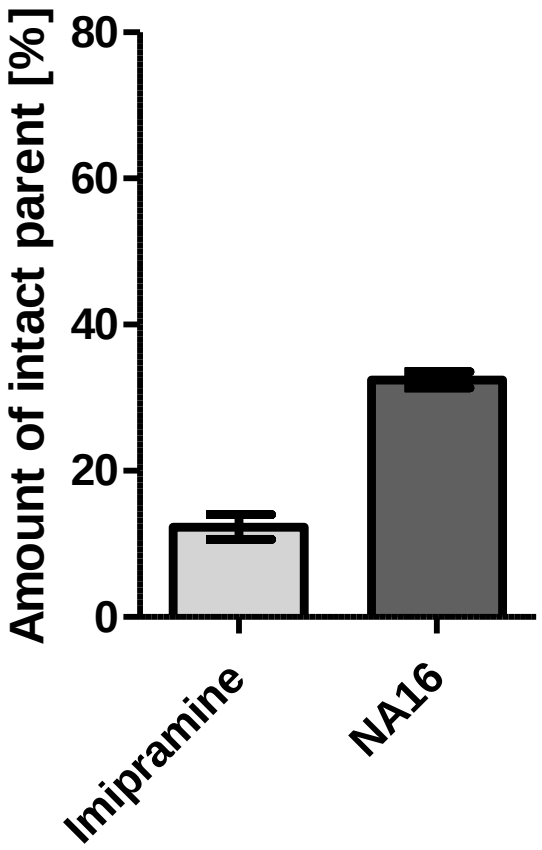
  - **c (NA16) > c (CX-4945)**
  - **NA16: Decrease of intracellular concentration**
  - **CX-4945: Increase of intracellular concentration**



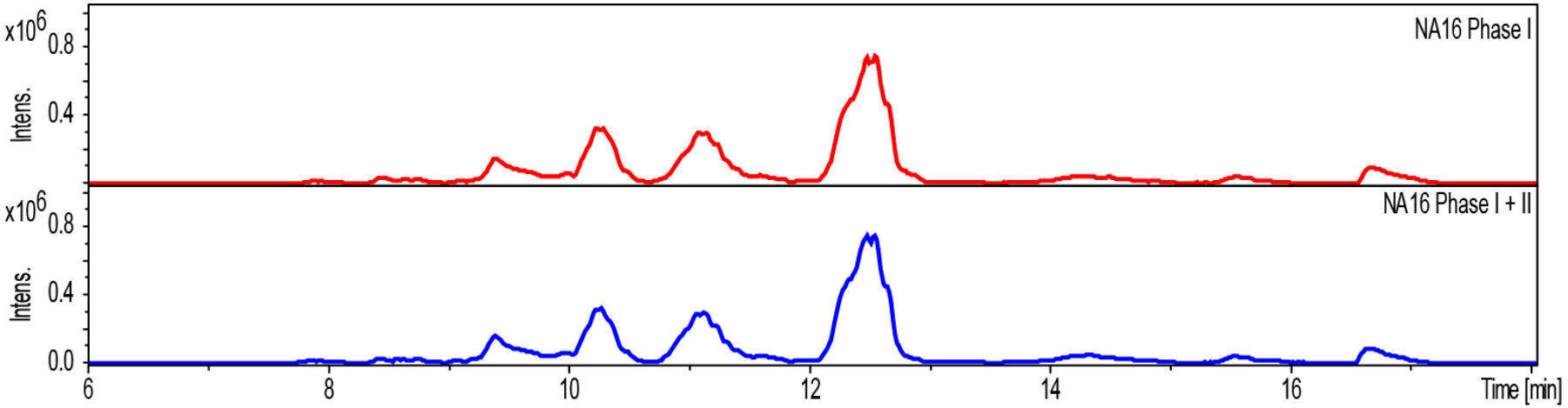
Results and discussion
4. Metabolism studies

- Investigation of metabolic stability of NA16:
  - Quantification of intact NA16 after incubation with phase I metabolism enzymes via HPLC-MS (Börgel *et al*. 2019)
  - Comparison with imipramine (control for metabolic labile substance)

- Amount of intact NA16 higher than amount of imipramine
  - ➢ **NA16 has a moderate metabolic stability**



Results and discussion
4. Metabolism studies



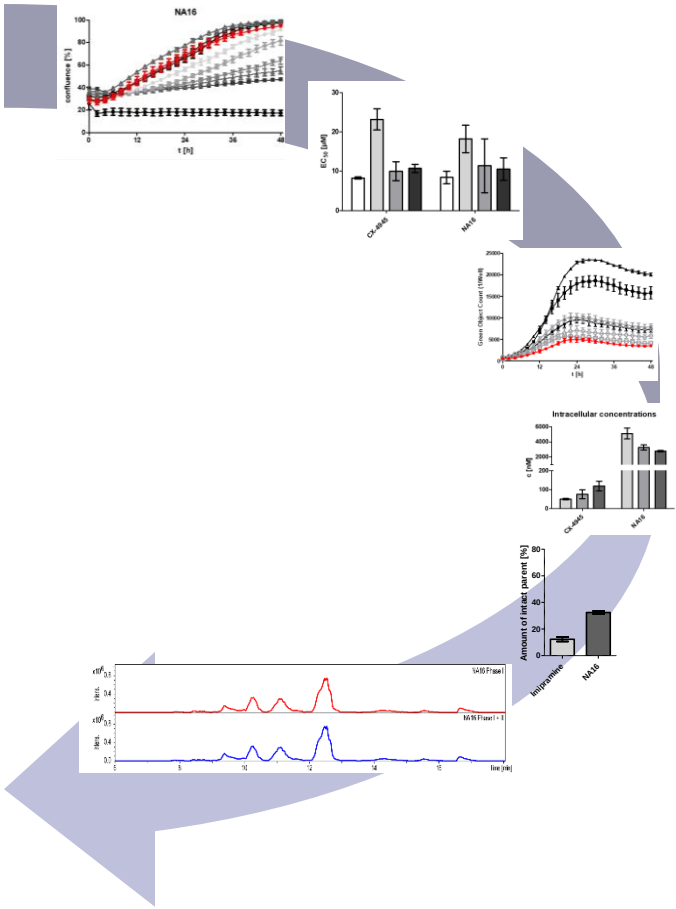
- Metabolite identification:
  - NA16 + phase I metabolism enzymes vs. NA16 + phase I & II metabolism enzymes (glucuronidation was investigated representative for phase II metabolism)
  - Analysis of metabolites via HPLC-MS (Börgel *et al.* 2019)

- Metabolites in both samples were equivalent
  - **No glucuronidation products of NA16 detectable**

Conclusions

- Effects of NA16:
  - Reduction of growth of A431, A549 and LNCaP cells
  - Influence on HUVEC growth comparable with influence of CX-4945
  - Induction of apoptosis weaker compared to treatment with CX-4945
  - Intracellular concentration higher than concentration of CX-4945; time-dependent decrease
  - NA16 is moderate metabolic stable; no glucuronidation products detectable

➢ **NA16 is worth analyzing further effects concerning its anti-tumor activity**



Acknowledgements

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