



# 6th International Electronic Conference on Medicinal Chemistry

1-30 November 2020

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## Targeting CK1 isoforms in colon and rectal cancer: initial steps towards the development of CK1 mutant-specific inhibitors

Joachim Bischof <sup>1,\*</sup>, Congxing Liu <sup>1</sup>, Lydia Witt <sup>2</sup>, Chiara Ianes <sup>1</sup>, Joana Baier <sup>2</sup>, Stefan Kirschner <sup>2</sup>, Doris Henne-Bruns <sup>1</sup>, Marko Kornmann <sup>1</sup>, Christian Peifer <sup>2</sup> and Uwe Knippschild <sup>1</sup>

<sup>1</sup> Department of General and Visceral Surgery, Ulm University Hospital, Albert-Einstein-Allee 23, 89081 Ulm, Germany

<sup>2</sup> Institute of Pharmacy, Christian-Albrechts-University of Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

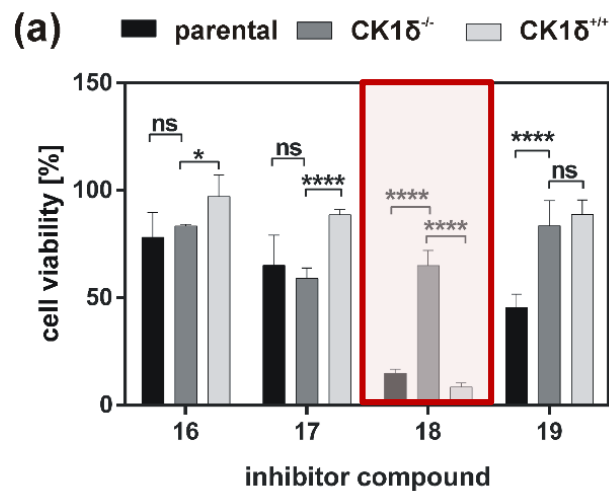
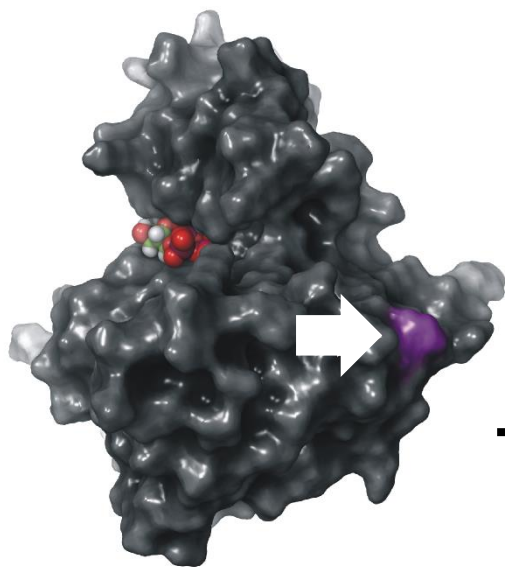
\* Corresponding author: [joachim.bischof@uniklinik-ulm.de](mailto:joachim.bischof@uniklinik-ulm.de)



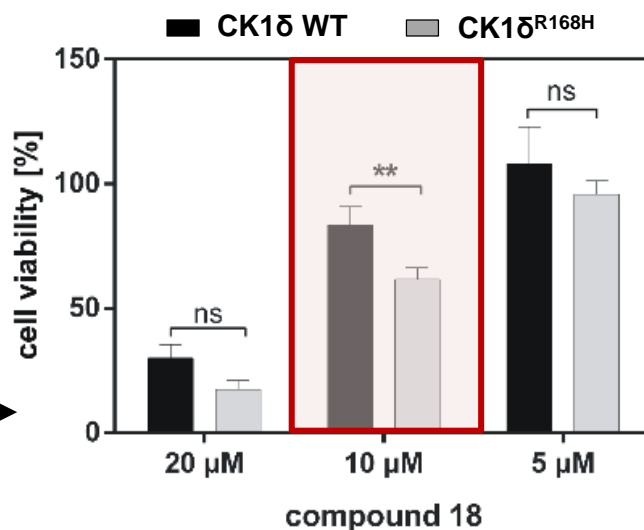
Christian-Albrechts-Universität zu Kiel

# Targeting CK1 isoforms in colon and rectal cancer: initial steps towards the development of CK1 mutant-specific inhibitors

CK1 $\delta$ <sup>R168H</sup>



HeLa cells



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## Abstract:

Colon and rectal cancer (CRC) represents the fourth leading cause of cancer related deaths among all neoplastic diseases. Dysregulation of expression and/or kinase activity of CK1 isoforms can be linked to tumorigenesis and oncogenic mutations in CK1 have previously been found in CRC patients. Therefore, inhibition of overexpressed or mutated CK1 isoforms is supposed to have promising potential for the treatment of CRC. In order to detect further hyperactive and potentially oncogenic CK1 mutants we first analyzed the kinetic properties of several CK1 $\delta$  mutants, which have been reported in different tumor entities. In subsequent experiments, we aimed at identifying small molecule inhibitors able to inhibit wild type and CK1 $\delta$  mutants and to affect the growth of established (tumor) cell lines either expressing wild type or mutant CK1 $\delta$ . In addition to well-established inhibitors of CK1 also newly developed compounds were tested, which are based on previously characterized IWP compounds (“inhibitors of Wnt production”). Among the tested molecules, inhibitors demonstrating CK1 isoform-specific as well as mutant-specific effects could be detected. Therefore, our results represent a starting point for further optimization approaches in the development of highly effective and specific CK1-targeting small molecule inhibitors.

**Keywords:** CK1; mutant; small molecule inhibitor; colon and rectal cancer; Wnt



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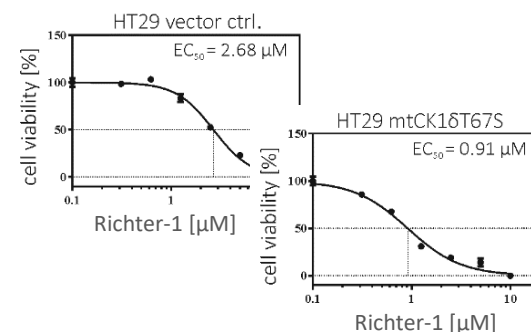
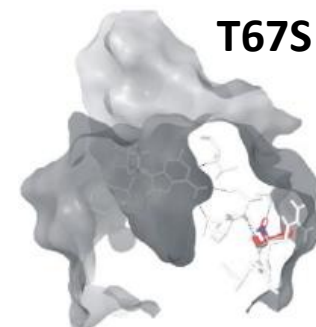
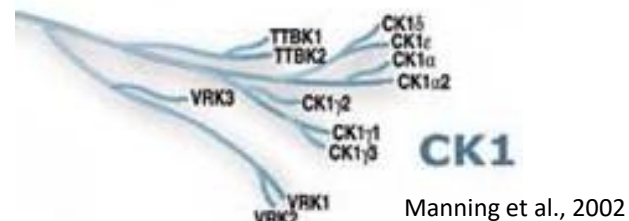
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# Introduction

- protein kinases of the **CK1 family**  
→ **regulation** of cell cycle, proliferation, and cellular differentiation
- **mutations** or altered **expression and/or activity**  
→ pathogenesis of a variety of tumors
- tissue of **colon and rectal cancer (CRC)**  
→ CK1 $\delta$  is **highly expressed** and CK1-specific inhibitors efficiently **block proliferation** of CRC cell lines

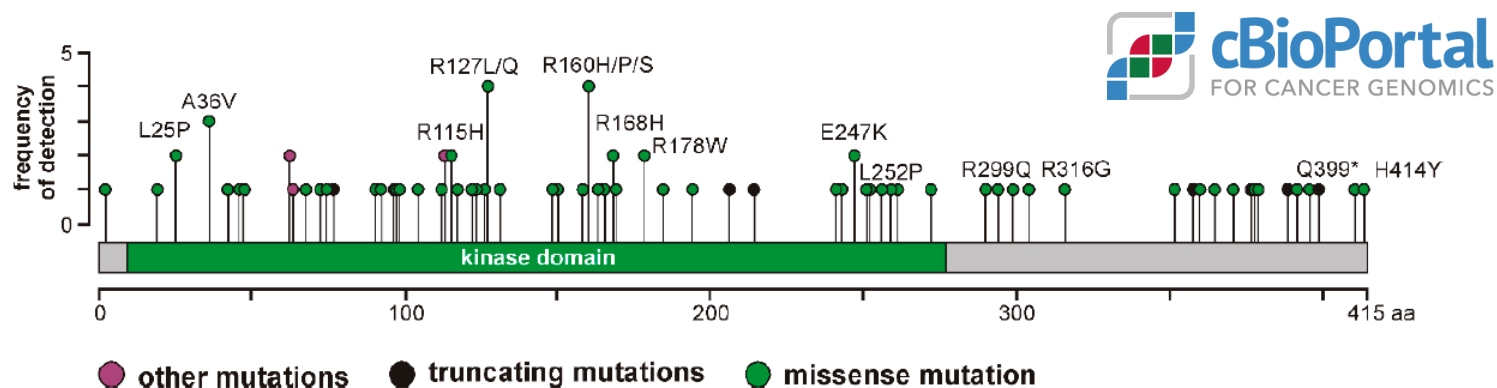


***CK1 $\delta$  as promising new target for novel therapeutic strategies!***



# Introduction

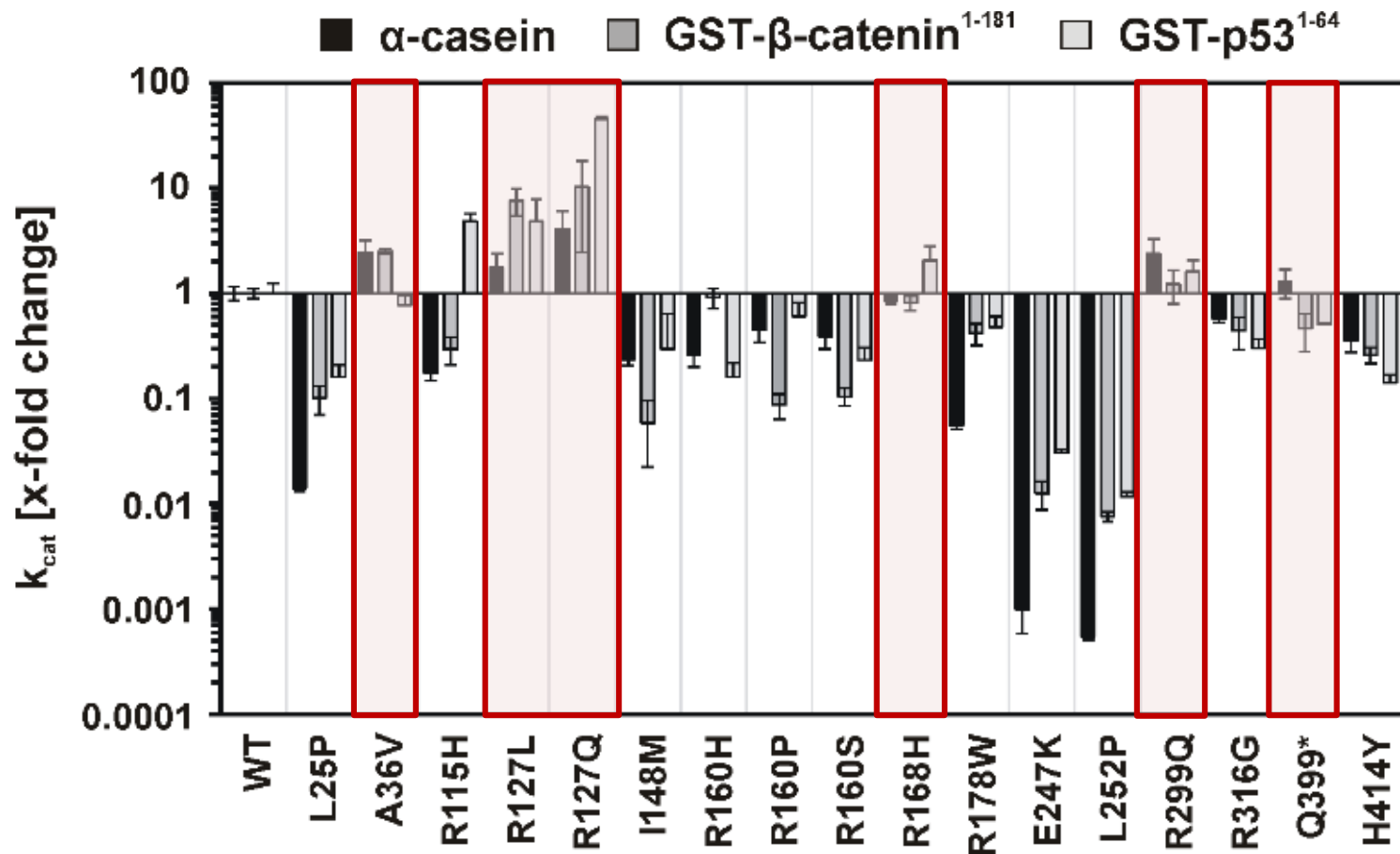
- identification of **CK1δ mutants** by database research



- determination of **kinetic parameters** for the different mutants
- analysis of **mutant-specific effects** of
  - (1) established and
  - (2) newly synthesized **CK1 small molecule inhibitors**



# Mutations in CK1δ result in altered kinetic parameters



# Hyperactive CK1δ mutants are more sensitive to different CK1-specific inhibitors (1)

| CK1δ variant | Bischof-5 | Richter-2 | IWP-2   | IWP-4   |
|--------------|-----------|-----------|---------|---------|
| WT           | 50 ± 17   | 50 ± 15   | 50 ± 6  | 50 ± 6  |
| A36V         | 43 ± 15   | 109 ± 33  | 55 ± 6  | 49 ± 7  |
| R115H        | 36 ± 10   | 211 ± 79  | 71 ± 34 | 46 ± 5  |
| R127L        | 50 ± 11   | 88 ± 34   | 42 ± 9  | 48 ± 10 |
| R127Q        | 24 ± 8    | 19 ± 2    | 37 ± 5  | 36 ± 11 |
| R168H        | 30 ± 16   | 31 ± 16   | 50 ± 9  | 61 ± 19 |
| R299Q        | 64 ± 22   | 69 ± 17   | 36 ± 20 | 91 ± 60 |
| Q399*        | 75 ± 30   | 50 ± 14   | 53 ± 27 | 96 ± 79 |

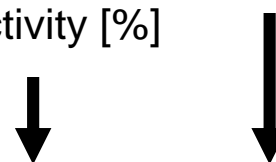
inhibition...

...similar to WT

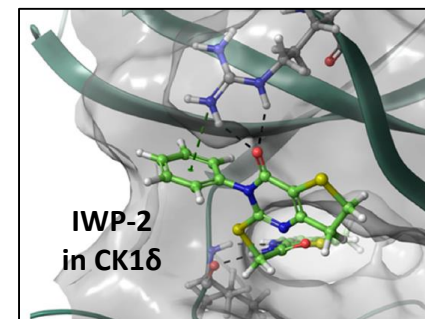
...stronger than WT

...less than WT

residual kinase activity [%]



synthesis of **new inhibitor compounds**  
based on previously characterized IWPs



García-Reyes et al., 2018



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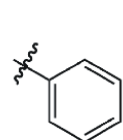
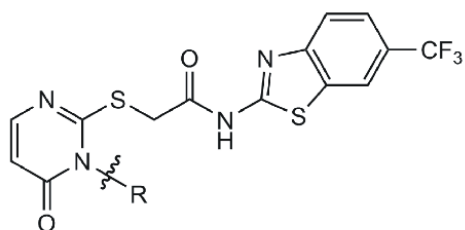
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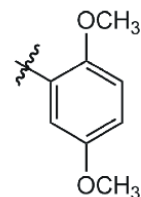


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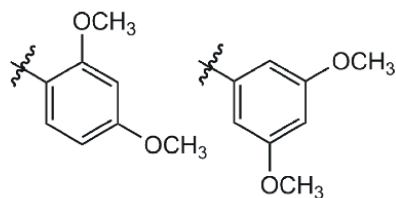
# Characterization of newly developed IWP-derivatives (2)



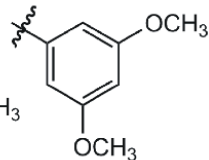
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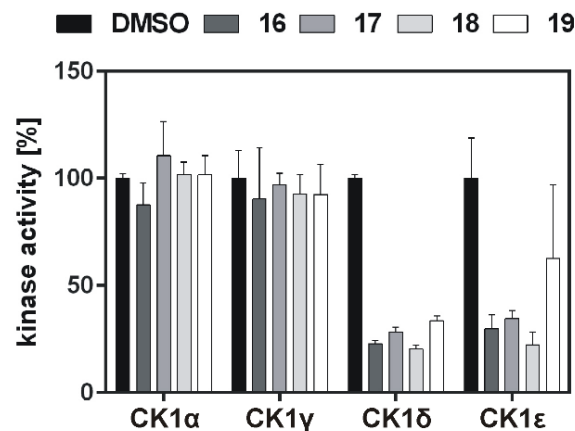
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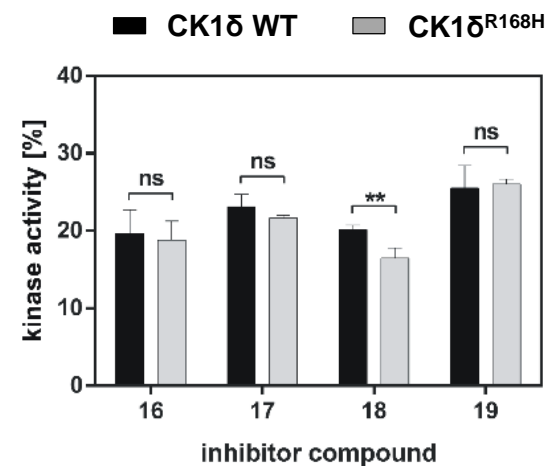
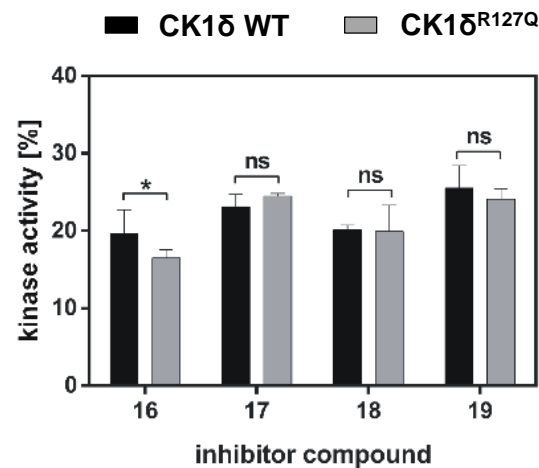
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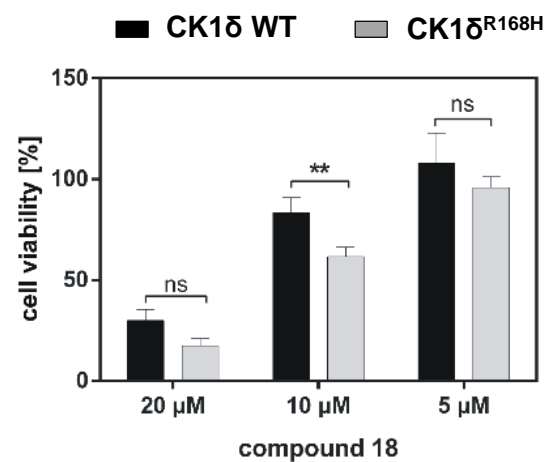
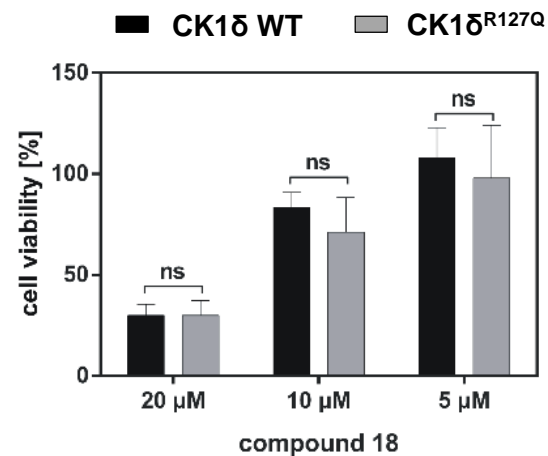
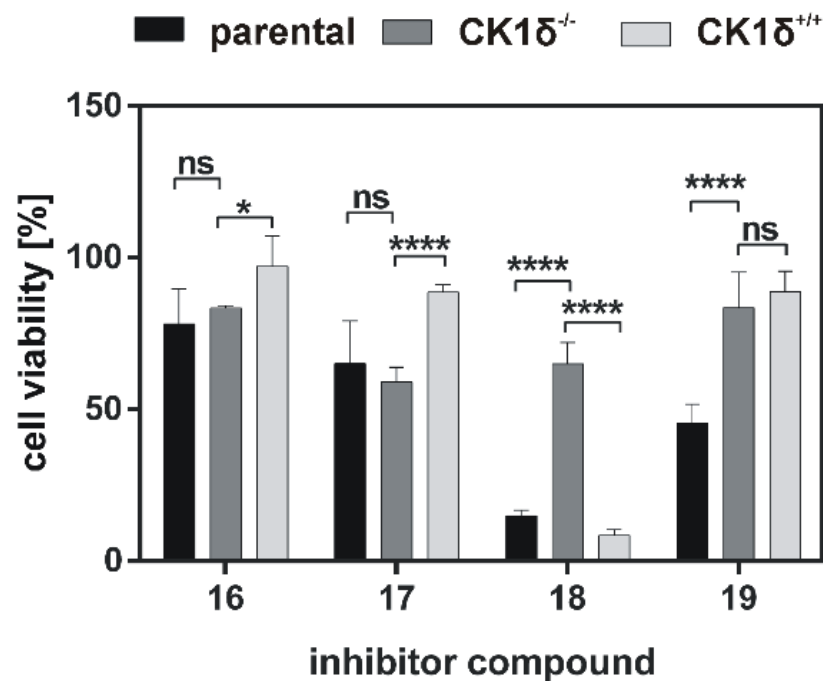
| inhibitor | IC <sub>50</sub> values for CK1δ [μM] |
|-----------|---------------------------------------|
| 16        | 2.0 ± 0.2                             |
| 17        | 3.5 ± 4.7                             |
| 18        | 1.7 ± 0.2                             |
| 19        | 4.7 ± 0.6                             |



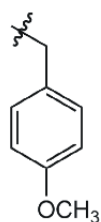
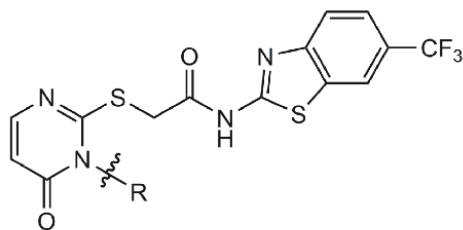


# Compound 18 selectively inhibits cellular CK1δ and shows stronger effects in cell culture

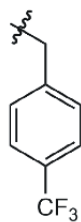
HeLa cells:



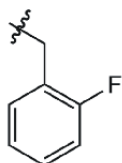
# A second set of shows improved inhibition of CK1δ...



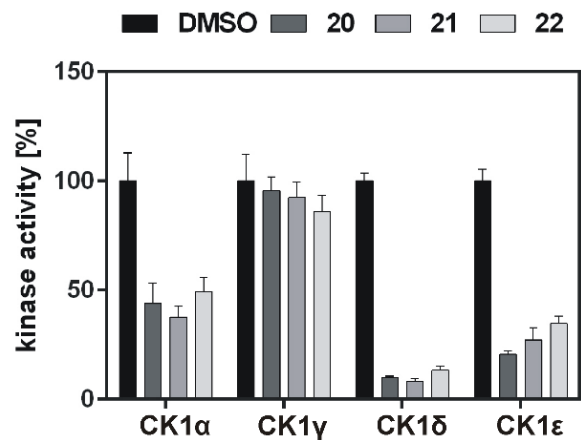
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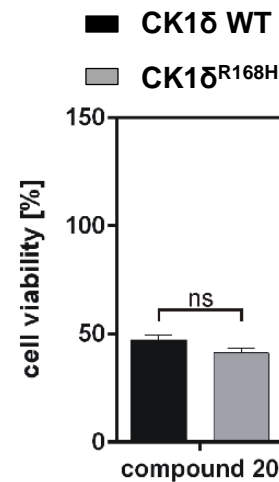
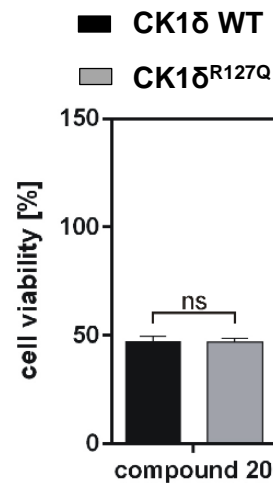
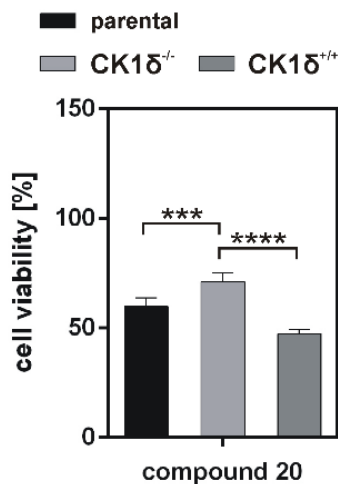
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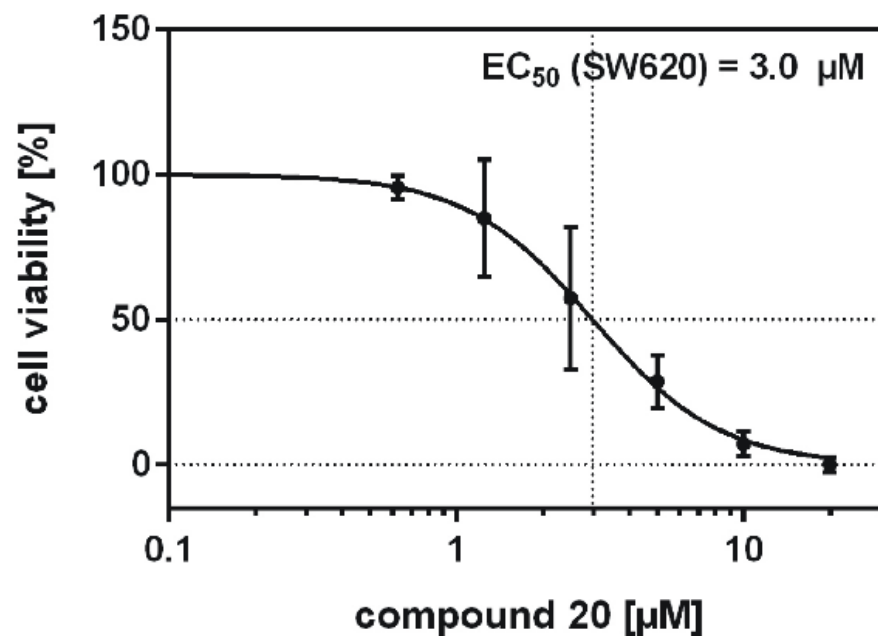
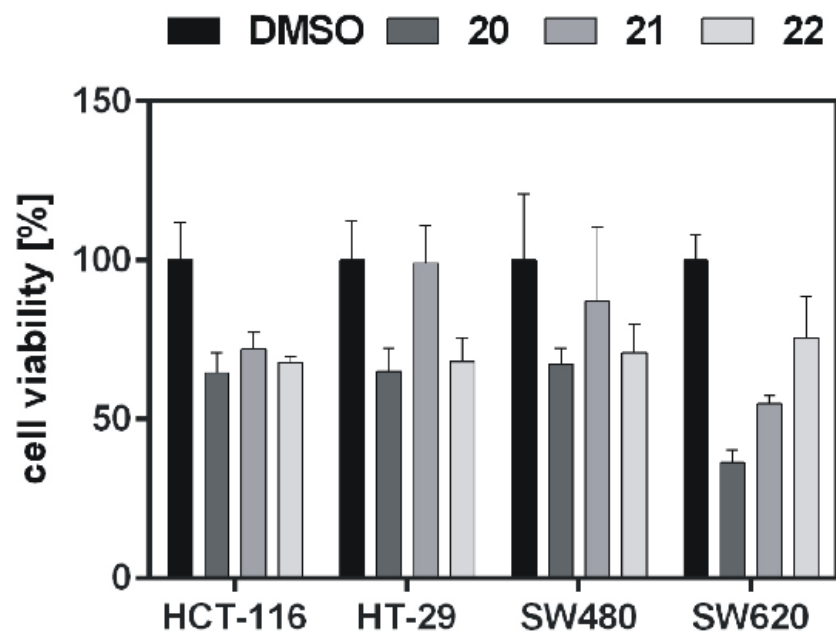
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| inhibitor | IC <sub>50</sub> values for CK1δ [μM] |
|-----------|---------------------------------------|
| 20        | 0.09 ± 0.01                           |
| 21        | 0.12 ± 0.02                           |
| 22        | 0.24 ± 0.04                           |



## ...and cell line-specific effects on colon cancer cell lines



# Conclusions

- what we already knew:
    - **mutations** or altered **expression/activity** of CK1 can be oncogenic
    - proliferation of cancer cell lines can be inhibited by **CK1 $\delta$ -specific inhibitors**
  - mutations in CK1 $\delta$  result in **altered kinetic parameters** *in vitro*
  - **CK1 $\delta$ <sup>R127Q</sup>** shows **stronger inhibition** by all tested (established) inhibitors
  - compound 18 shows **mutant-specific inhibitory potential** (CK1 $\delta$ <sup>R168H</sup>)
  - **mutant-specific effects** and **efficacy in cell culture** need to be improved
- kinetic analysis **broadens our knowledge** about the (tumorigenic) functions of CK1 isoforms
- potential use of CK1 $\delta$ -specific inhibitors in **new therapeutic strategies** for the treatment of proliferative disorders



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Dr. Lydia Witt  
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**Newly Developed CK1-Specific Inhibitors Show Specifically Stronger Effects on CK1 Mutants and Colon Cancer Cell Lines.** Liu C, Witt L, Ianes C, Bischof J, Bammert MT, Baier J, Kirschner S, Henne-Bruns D, Xu P, Kornmann M, Peifer C, Knippschild U. Int J Mol Sci. 2019 Dec 7;20(24):6184. doi: 10.3390/ijms20246184.



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