Real-time quantitative PCR based analysis of transcriptional effects of CDK8/Cyclin C inhibitors

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Introduction

The CDK8/Cyclin C complex regulates the transcription of genes via different pathways. The best understood role is its function as member of the Mediator complex by phosphorylation of the C-terminus of RNA polymerase II and by direct phosphorylation of transcription factors such as STAT1. CDK8 has been identified as an oncogene being amplified in different types of human cancer. In this study, we compared a type I (CCT251545[1]) and type II (MC116, Reaction Biology) CDK8/Cyclin C inhibitor in biochemical, cellular, and in vivo assays. In addition, we analyzed their effects on the expression of selected genes controlled directly or indirectly by CDK8/Cyclin C, namely c-myc, cyclinD1, BCL-XL, and XIAP[2,3,4] by real-time quantitative PCR (qPCR) in cells and in vivo.

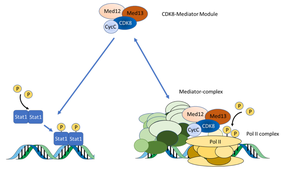


Fig. 1: Schematic depiction of CDK8/CycC function in the regulation of transcription as member of Mediator and by direct phosphorylation of transcription factors like STAT.

Biochemical Characterization

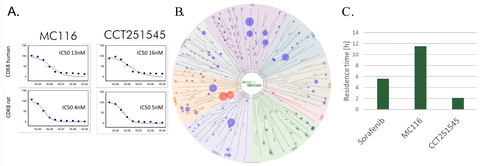


Fig. 2: Biochemical characterization of the inhibitors
(A) Reaction Biology's type I inhibitor MC116 shows comparable potency as reference compound type II inhibitor CCT251545 in biochemical activity assays against both human and rat CDK8/CycC complexes.
(B) The selectivity profile of MC116 shows high inhibition against CDK8 and CDK19. Diameters of circels represents % inhibition at test concentration. Biochemical testing was performed with the radiometric ^{33}PanQinase activity assay format.
(C) The residence time of MC116 is about 12 h and thus 5 times longer than that of CCT251545, about twice as long as type II inhibitor sorafenib. The residence time was measured by reporter displacement assay[5].

Cellular Characterization

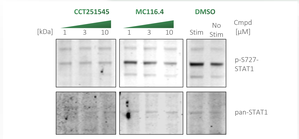


Fig. 3: MC116.4 as well as CCT251545 inhibits STAT1 phosphorylation in a cell-based assay.
HCT116 cells were incubated with three different doses of indicated compounds for 1.5 h with subsequent stimulation with IFNg (100ng/ml, 30min). Cells were lysed, and p-S727 STAT1 as well as STAT1 was detected by western blotting as indirect readouts of CDK8 activity. (MC116.4 = MC116-Tosylate salt)

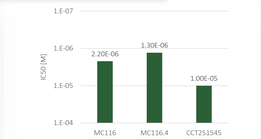


Fig. 4: Moderate inhibition of viability of human HCT116 colon cancer cells by treatment with MC116, MC116.4, and CCT251545.
Cells were treated with 8 different concentrations of MC116; MC116.4, and CCT251545. Viability was measured after 72 h using CellTiterGlo (n ≥ 2).

In vivo Characterization

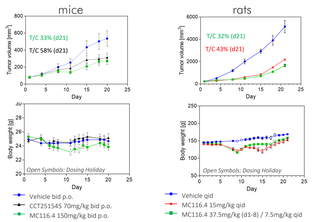


Fig. 5: MC116.4 significantly inhibits the growth of human colon xenograft tumors in mice and rats
HCT116 cell were implanted s.c. in nude mice and nude rats, respectively. Once tumors were established treatment with MC116.4 (mice and rats) and CCT251545 (mice) were performed as indicated.

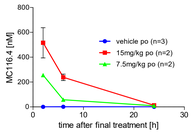


Fig. 6: Oral administration of MC116.4 in tumor bearing mice results in dose dependent plasma exposure
Plasma were collected from animals from efficacy study (confer Fig. 5) after 1, 6, and 24 h after final dosing with indicated MC116.4 doses.

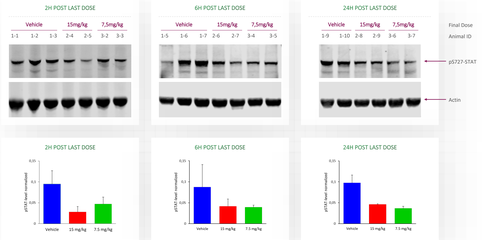


Fig. 7: MC116.4 treatment reduces pS727-STAT1 levels in HCT116 tumors grown in nude rats up to 24 h after final dose.
Tumors were collected at different time points after final dose, and pS727-STAT1 was detected by western blotting. Lower panel represents the quantiative analysis of the western blot signals shown in the upper panel.

qPCR Analysis

Fig. 8: MC116.4 and CCT251545 show different effects on the expression of four CDK8/CycC dependent genes in HCT116 colon cancer cells.
Analysis of gene expression levels of Bcl-xL (A), XIAP (B), Myc (C), and CycD1 (D) by qPCR. Data were analyzed by the ΔΔCt method using GAPDH expression for normalisation. Each column represents the mean ±SE of two biological replicates. HCT116 cells were starved overnight, pretreated for 1 hrs with different concentrations of compounds or 0.1% DMSO, and stimulated for 2 hrs with 10% FCS.

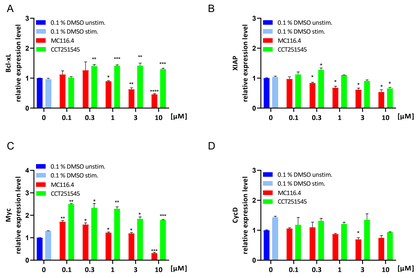


Fig. 9: HCT116 tumors grown in nude mice show differential expression of CDK8/CyclinC/Mediator-dependent genes in response to CDK8 inhibitors CCT251545 and MC116.4 treatment.
Analysis of gene expression levels of Bcl-xL (A), XIAP (B), Myc (C), and CycD1 (D) by qPCR. Data were analyzed by the 2-ΔΔCt method using GAPDH as a reference gene. Each column represents the mean ±SE (n=6). MC116.4 and CCT251545 treatment was done as described in Fig 5. Tumors were harvested 24 h after final treatment.

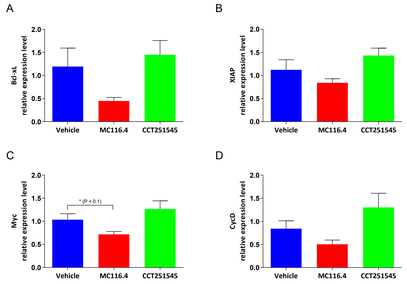
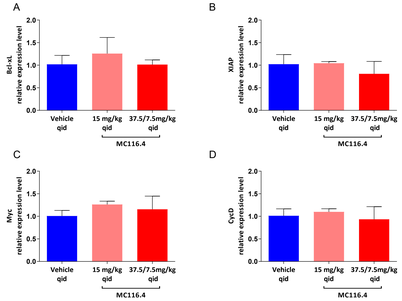


Fig. 10: HCT116 tumors grown in nude rats did not show significant differential expression of CDK8/CyclinC/Mediator-dependent genes in response to MC116.4 treatment
Analysis of gene expression levels of Bcl-xL (A), XIAP (B), Myc (C), and CycD1 (D) by qPCR. MC116.4 and CCT251545 treatment was done as described in Fig. 5. Tumor harvested 24 h after final treatment were analysed. Data were analyzed by the 2-ΔΔCt method using GAPDH as a reference gene. Each column represents the mean ±SE (n=2).



Summary

1. Type II inhibitor MC116 efficiently inhibits CDK8/CycC in biochemical assay with similar potency as type I reference inhibitor CCT251545.
2. Cellular potency as quantified via inhibition of STAT1 phosphorylation on S727 was detectable with both compounds, but more pronounced by CCT251545.
3. Inhibitors MC116 (and tosylate salt MC116.4) as well as CCT251545 show moderate inhibition of viability of HCT116 cell after 72 h incubation.
4. Direct comparison of both CDK8/CyclinC inhibitors MC116.4 and CCT251545 indicate similar potency in a human HCT116 xenograft model in nude mice.
4. MC116.4 efficiently inhibits tumor growth in a human HCT116 xenograft model in nude rats.
5. MC116.4 treatment resulted in more pronounced reduction of mRNA expression of BCL-XL, XIAP, Myc, and CycD1 compared to CCT251545 in HCT116 cells as well as in HCT116 xenograft tumor grown in mice.
6. No differences in the expression BCL-XL, XIAP, Myc, and CycD1 upon treatment of HCT116 xenograft grown in rats could be detected 24 h after final dose of rats with MC116.4.

References

1. Dale et al 2015: Nature Chemical Biology 12
2. Firestein et al 2008: Nature 455
3. Rolinski et al 2021: Nucleic Acid Research 49
4. Kuuluvainen et al 2018: Molecular and Cellular Biology 38
5. Neumann et al 2011: Methods in Enzymology 493

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