DEVELOPMENT OF BIOCONJUGATES AND THEIR MODUL CONSTRUCTS FOR TARGETED THERAPY OF CANCERS WITH HIGH MORTALITY

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The *in vitro* antitumor effect of ONC201 derivatives in pancreatic and in colorectal cancer cell lines

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Introduction

ONC201 (also called TIC10–TRAIL-inducing compound) is a new drug candidate that belongs to the family of small molecules.[1] It is water soluble, lipophilic (logP=2.3); thus it can be administered *per os* which is an important factor in the adherence of patients.[1] It is currently investigated in early-phase clinical trials aiming to cure solid tumors.

Although the mechanism of ONC201 is still not clear, from literature we know that it may be able to activate both intrinsic and extrinsic apoptosis.[2]

It can trigger apoptosis via p53-dependent ways by increasing the activation of caspases.[3] By inhibiting the Akt and MAPK intracellular pathways independently from the p53-status of the cells, the FOXO3a transcription factor dephosphorylates and its activation increases.[4] Due to the change in the conformation of this protein, it can easily penetrate into the nucleus, where it enhances the expression of the TRAIL protein (TNF-related apoptosis-inducing ligand). The TRAIL protein can then trigger apoptosis selectively in cancer cells upon the greater presence of TRAIL-R1 and TRAIL-R2 in the surface membrane of cancerous cells.[5]

The reference molecule, ONC201 can be modified with ferrocene, benzyl and halogenated benzyl group on the heterocyclic skeleton in order to optimize the pharmacokinetics of the molecule and to increase the antitumor effect (Figure 1).

Objectives of the present study were:

1. to study the antitumor effect of ONC201 and its 30 newly synthesized derivatives in PANC-1 and COLO-205 cell lines
2. to compare the efficacy of the derivatives with the parent molecule
3. to select the most effective 3 derivatives for further cell cycle analysis

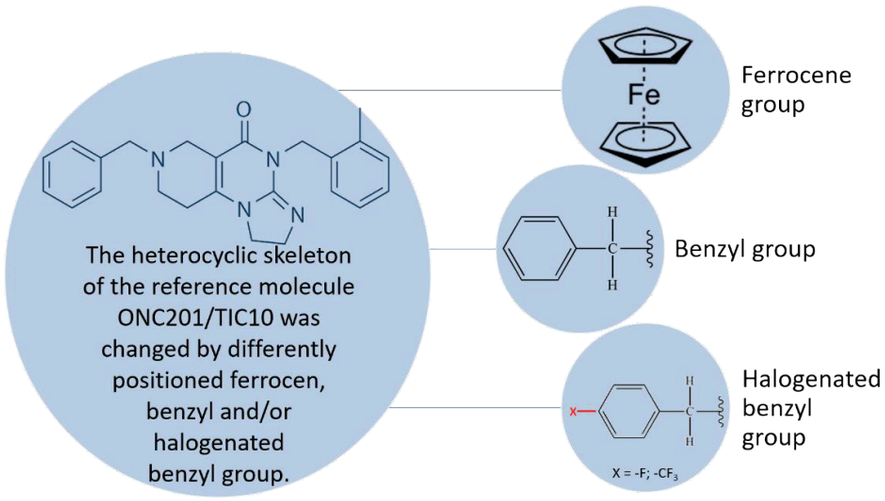

Figure 1. Groups of investigated TIC10 derivatives

Results

After evaluation of the cell viability assays, the IC_{50} value of the reference molecule was not detectable on PANC-1 cells, but on COLO-205 it was 5 µM after a 72h long incubation (Figure 2). Table 1. shows that three derivatives were identified as more potent candidates than the reference molecule upon their lower IC_{50} values. The most potent derivates all belong to the group modified with a halogenated benzyl group.

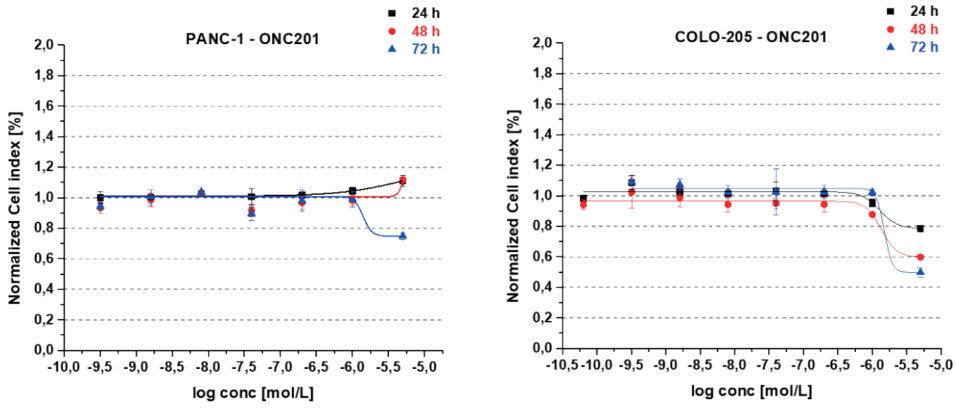

Figure 2. Normalized data and fitted dose-response curves of the reference molecule for PANC-1 and COLO-205 cell lines

As the upregulated TRAIL protein can act through the activation of death receptors, e.g. the TRAIL-R1 and TRAIL-R2, the status of the cell lines on these two receptors was determined by flow cytometry. The COLO-205 and the PANC-1 cells also seem to express both of the receptors, but COLO-205 cells tend to have a higher percentage of TRAIL-R1 and TRAIL-R2 than the PANC-1cells (Figure 3).

Table 1. IC_{50} values (μM) after 72 hours of treatment

	PANC-1	COLO-205
ONC201 (ref. molecule)	ND	5
Molecule 1	0.77	0.21
Molecule 2	0.16	0.25
Molecule 3	0.35	0.25

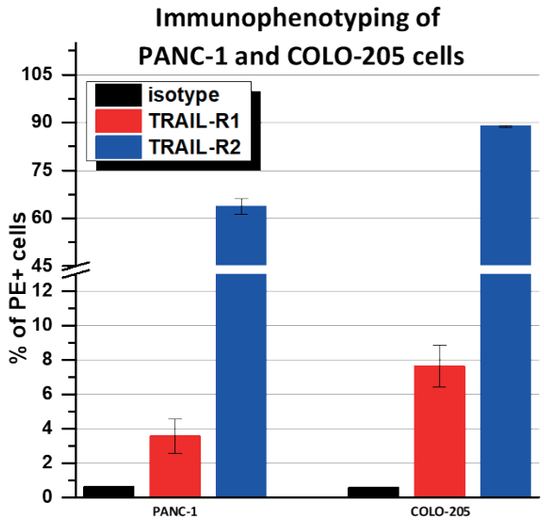

Figure 3. Levels of expression of TRAIL-R1 and TRAIL-R2 on the surface membrane of PANC-1 and COLO-205 cells

For further cell cycle analysis, the compounds were tested at 1.5 μM concentration. This concentration is the IC_{20} value of the tested reference molecule for both tumorous cell lines. ONC201 and its 3 derivatives had a barely significant effect on the cell cycle phase distribution of PANC-1. In COLO-205, an increase in the number of the cells in the subG1 phase was detected after 48h long treatment. This increase correlates with the decrease of the cells in the G_0/G_1 phase. This idea suggests that the compounds can act independently from the cell division and can target resting cells in the G_0 phase (Figure 4).

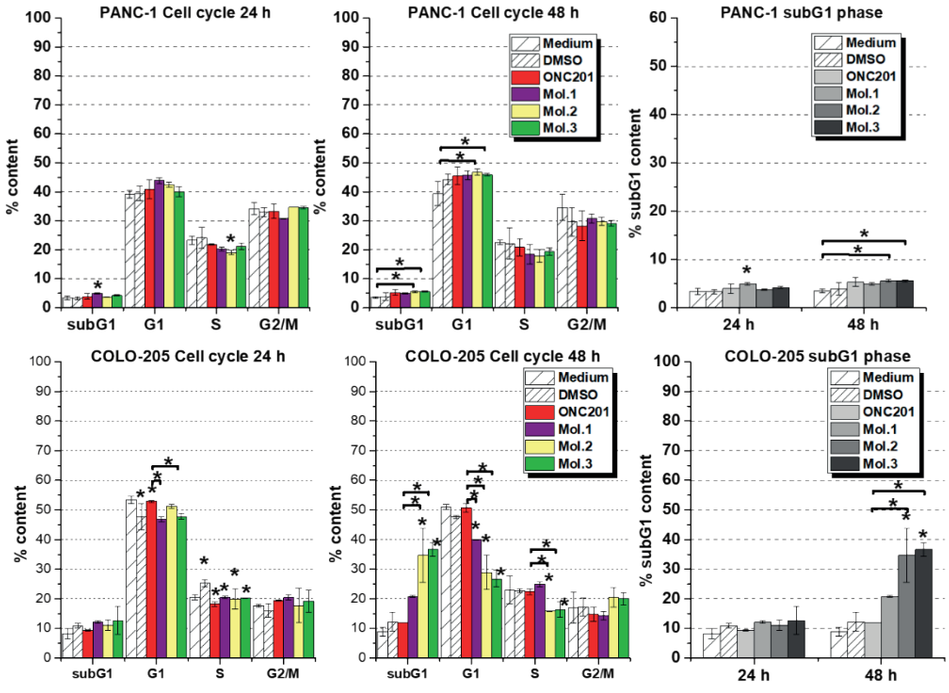

Figure 4. Cell cycle profile of PANC-1 and COLO-205 cells after 24 and 48 h long treatment with the different compounds at a concentration of 1.5 μM

Conclusion

Our results show that ONC201 had a more potent effect on the colorectal tumor cell line COLO-205 than on the pancreatic adenoma cells PANC-1. Our results have a good correlation with the previous results of the literature.[6] All of the tested compounds induced cell cycle arrest in the cell lines after 48 hours of the treatments, although no decrease in the cell viability was measured at this time point, which results confirm previous findings of the literature.[7] As the COLO-205 tumorous cell line expresses a higher percentage of TRAIL-R2 than TRAIL-R1, our theory is supported that ONC201 can trigger cell death *via* the activation of TRAIL-R2.

In conclusion, our data suggest that the investigated derivatives are potentially good candidates for antitumor therapy of colorectal cancers in the future.

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