IN VITRO EVALUATION OF SMALL MOLECULES AND PEPTIDE-BASED CONJUGATES FOR TARGETED TUMOUR THERAPY IN PANCREATIC TUMOUR CELL LINES

PhD thesis

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers, with a five-year survival rate of just 11%. This poor prognosis is primarily due to late-stage diagnosis and the tumour's resistance to chemotherapy, which often worsens over time. Given these challenges, there is an urgent need for more effective treatment strategies.

Targeted therapies offer hope for more effective treatments with fewer side effects. These therapies fall into two main categories: small molecules and macromolecules/biologics. A notable development in small-molecule therapy is the imipridone class, particularly ONC201 - also known as TRAIL (TNF-related apoptosis ligand)-inducing compound 10 (TIC-10) - which is being explored in clinical trials.

Beyond single-agent therapies, combination treatments are a well-established strategy in oncology, offering a way to amplify anti-tumour effects, overcome drug resistance, and reduce toxicity through dose optimization. The combination of ONC201 with other drugs is already under investigation, aiming to enhance its therapeutic potential. Integrating combination regimens may hold the key to more effective PDAC treatment in the future.

Another emerging strategy involves peptide-drug conjugates (PDCs), a class of macromolecular therapeutics that serve as specialized drug delivery systems. PDCs consist of a targeting peptide sequence linked to a drug molecule, either directly or via a linker peptide. In this thesis, daunorubicin (Dau), an anthracycline, was selected as the cytotoxic agent within the PDC framework to enhance drug selectivity and effectiveness in treating PDAC.

2. Objectives

In my PhD, I studied the significance of halogenation on anti-tumour effects of ONC201 and PDCs. I investigated the role of fluorination in enhancing the pharmacological activity of meta- and para-fluorinated ONC201 analogues using the PANC-1 PDAC cell line. Additionally, I explored the molecular synergy between ONC201 and bortezomib in metastatic melanoma cells (A2058).

a. Do the analogues have anti-tumour effects on the tumour cell line, and do they have a direct cytotoxic effect on PANC-1 cells?
b. Do they influence the cell cycle of PANC-1 cells?
c. Do they induce apoptotic cell death in PANC-1 cells?
d. Are they able to induce the level of TRAIL in PANC-1 cells?
e. What is the molecular background of their supposed apoptosis-inducing effect in PANC-1 cells?
f. Do the combination of ONC201 and bortezomib increase the expression of the death receptors on metastatic melanoma, A2058 cell line?

The second part of my thesis examines how incorporating non-natural amino acids, like halogenated amino acids, into the targeting sequence of Dau-peptide conjugates affects PANC-1 cell viability and death.

a. Do the conjugates have anti-tumour effects on the tumour cell line, and to what extent can the PANC-1 cells uptake the conjugates?
b. Do they have a direct cytotoxic effect on PANC-1 cells?
c. How can they mediate their anti-tumour effect on PANC-1 cells?
d. Do they have cardiotoxic effects in vitro in the long term?

3. Methods

3.1. Cells and materials

Two tumour cell lines (pancreas adenocarcinoma, PANC-1 and metastatic melanoma, A2058) were used in our experiments to evaluate the effects of the investigated molecules on cell physiological parameters. To investigate the tumour selectivity of the ONC201 derivates, normal human dermal fibroblast cells (NHDF) were used. To test the cardiotoxicity of the tested conjugates, an immortalised mouse cardiomyocyte cell line (HL-1) was used.

During our experiments the cells were treated with ONC201 and its analogues or with bortezomib and its combinations or with the Dau containing PDCs. The ONC201 and its analogues were synthesized by Professor Antal Csámpai and Péter Bárány, and the peptide conjugates were prepared by Professor Gábor Mező and Nóra Kata Enyedi at the Institute of Chemistry, Faculty of Science, Eötvös Lóránd University, and were kindly provided to us for testing. The stock solutions were aliquoted and stored at -80°C, and fresh dilutions were prepared using a complete cell culture medium for each experiment.

3.2. Viability assay

In the case of ONC201 and its derivates, the aim of the cell viability measurements was to determine the half-maximal inhibitory concentration (IC_{50}) value at which the treatment reduced the cell viability to 50% compared to the control-treated samples. We have tested the molecules in a 2-fold dilution series ranging from 50 μM to 250 nM. For testing the cell viability after treatment with Dau and the peptide conjugates, three different concentrations (1, 10 and 100 μM) were used.

3.2.1. xCELLigence SP

The xCELLigence SP device works based on impedimetric and real-time measurement techniques. The effects of the molecules on the viability of PANC-1 cells were determined using this system. Firstly, a baseline was obtained with a cell-free culture medium for 1 hour at 1-minute intervals. Then, the cells were added to the so-called E-plate. After 24 hours, the investigated compounds were added to the system, and the changes in impedance were monitored for 96 hours. The Cell Index (CI) was used to express the changes in impedance as a relative and dimensionless parameter.

3.2.2. AlamarBlue-assay

The AlamarBlue assay was used to measure the viability of NHDF cells. First, the cells were plated then after 24 hours of cultivation, the cells were treated with the tested compounds. After 24, 48 and 72 hours of treatment, AlamarBlue (0.15 mg/mL resazurin) was added to the cells, and after 5 hours of incubation with the dye, the fluorescent intensity of the samples was measured (λex: 530-560 nm, λem: 590 nm) with the Fluoroskan FL fluorimeter.

3.2.3. CellTiter-Glo

In order to determine the cardiotoxic effects of Dau and the peptide conjugates, the CellTiter Glo Luminescent Cell Viability Assay was used to measure the viability of HL-1 cells. When the spontaneous pacemaker activity of the cells started, after 72 hours of culturing, 100 µL fresh media was added and then treated the cells with the conjugates (ConjA, Conj03 and Conj16) at 1, 10 and 100 µM concentrations for 72 hours. Then, we followed the manufacturer's instructions and the Fluoroskan FL fluorimeter was used to measure the luminescence.

3.3. Apoptosis assay

To determine the number of cells in the early- and late apoptotic stages a double labelling method was used for flow cytometric analysis. In the case of ONC201 and its derivatives, 7AAD (7-Aminoactinomycin D) was used, and after treatment with Dau and the peptide conjugates, To-Pro^{TM}3 was used in combination with Annexin V- FITC. In the case of treatment with ONC201 and its analogues, the cells were treated at concentrations of 0.5, 10 and 25 μM for 72 hours. While in the case of the conjugates, the cells were treated for 48- and 72 hours at 10 μM. BD FACSCalibur was used for the flow cytometric analysis. The data was evaluated using Flowing Software 2.5.1.

3.4. Cell cycle analysis

To analyse the cell cycle, the DNA content of cells was measured with a flow cytometer (BD FACSCalibur) using propidium iodide (PI). After 48- and 72-hour treatment with 0.5 μM ONC201 and its analogues, the cells were prepared and resuspended in 70% ethanol at -20℃ and incubated for 30 min at room temperature, followed by an additional 24-hour incubation at -20℃. Then, to remove the ethanol, the cells were centrifuged for 5 min (300 *g*). Subsequently, the cells were resuspended in citric acid/ disodium hydrogen phosphate buffer (pH=7.8) containing 100 μg/mL RNase A. 6 μL of PI was added immediately before measurement to each sample. To evaluate the results, Flowing Software 2.5.1 was used.

3.5. Direct cytotoxicity assay

The CyQuantTM LDH Cytotoxicity Assay was used to determine the direct cellular cytotoxicity of the investigated compounds. After 24 hours of cultivation, the cells were treated with the molecules. 50 μL of the media from each well was pipetted into a new 96-well plate after 48 and 72 hours of

treatment. The assay was then performed according to the manufacturer's manual. The absorbance was measured for each well at 492 and 680 nm using a plate reader. The '% cytotoxicity' was then calculated according to the manufacturer's instructions.

3.6. Tracking of the morphological changes

To track the morphometric changes of PANC-1 cells after treatment, a holographic transmission microscope was used. After 24-, 48- and 72-hour treatment, at least 5 images were taken from each culture dish, making sure to assess different fields of view. During the image analysis three parameters (cells' average area, optical thickness and optical volume) were tracked and analysed by the built-in software. For the analysis, a minimum of 25 cells were identified in each image using the minimum error histogram-based threshold algorithm in the software.

3.7. Apoptotic protein analysis

The expression of 35 apoptosis-related proteins were determined in PANC-1 cells treated with ONC201 and its analogues (0.5 μM, 72 h) using the Human Apoptosis Array Kit. The total protein content was isolated from the cells and measured using the Micro BCA Protein Assay Kit according to the manufacturer's protocol. 225 μg of protein was added to the membranes, and the required steps were carried out in accordance with the manufacturer's instructions. The Bio-Rad Chemidoc XRS+ instrument was used to take images of the membranes and the images were analysed using ImageLab software.

3.8. Gene expression analysis

The total RNA was isolated using the RNeasy kit according to the manufacturer's description. RNA was then reverse transcribed into cDNA at a concentration of 1000 ng/20 μL using the SensiFAST™ cDNA synthesis kit.

For the amplification, the Sso Advanced Universal SYBR Green Supermix was used. To calculate the changes in gene expression, the $2^{-(\Delta\Delta Ct)}$ method was used. Upregulation was considered biologically significant with at least a 2-fold change, while downregulation was defined as at least a 0.5-fold change.

To determine the effect of bortezomib and ONC201 and their combinations on the expression of death receptors in A2058 cells, *DR4* and *DR5* at the mRNA level were determined after 72-hour treatment.

To test the effects of ONC201 and its analogues on the apoptotic gene expression of PANC-1 after 3-, 6-, 12-, 24-, and 48-hour treatment, 4 target genes were selected: *DR4* and *DR5*, *p53* and *TRAIL*.

In the case of PDCs, first, the changes in the expression of 88 genes related to cellular senescence were screened using a predesigned 96-well panel (Cellular senescence (SAB Target List). In this screening, a 48-hour treatment of 10 μM Conj16, the most effective conjugate, was studied on PANC-1 cells. 14 genes *GLB1, CDKN1A, TP53, TP53BP1, E2F1, E2F2, E2F3, TERT, CCNA1 CDC25A, CDC25C, CCND2, CCNE1, LGALS3, TBP* were selected for further analysis and comparison of the effect of the three selected conjugates (ConjA, Conj03, Conj16) based on the results of the screening.

3.9. Cellular uptake of the peptide-drug conjugates

To determine the cellular uptake of the Dau-peptide conjugates to PANC-1 cells, a Cytoflex flow cytometer was used. After 24 hours of cultivation, the cells were treated with Dau (0.5, 1 μM) and with the conjugates at 10 μM for 1 hour at 37 °C and 4 °C simultaneously. The CytExpert Software was used for data evaluation. The quantification of the relative amount of the conjugates taken up by the cells was calculated from the geometric mean value of the fluorescence intensity of Dau (conjugated or unconjugated) in the sample.

3.10. Statistical analysis

Origin Pro8.0 software was used to statistically evaluate the data (mean ± SD of parallel measurements) presented in the Results section -. The IC_{50} value was calculated by plotting the viability values as a function of log concentrations, fitting a sigmoidal dose-response curve to the points. A one-way analysis of variance (ANOVA, *post hoc* test: Fisher's LSD) was used to analyse the significance of the data. Levels of significance were indicated as follows: x - $p<0.05$; y - $p<0.01$; z - $p<0.001$.

4. Results

4.1. The effect of the small molecule, ONC201 and its fluorinated analogues

4.1.1. The fluorinated analogues have a reduced IC_{50} on PANC-1 cells and spare the healthy NHDF cells.

After 24 and 48 hours of treatment, no viability-reducing effect was detected for any compounds on PANC-1 cells. In the case of ONC201, the IC_{50} value after 72-hour treatment was 6.1 μM. With the introduction of fluorine into the demethylated ONC201 structure (TBP-134, TBP-135), the IC_{50} value significantly decreased to 0.35 and 1.8 μM, respectively.

To determine the tumour-selective effect of the molecules, the viability of NHDF cells was measured. Although no significant antiproliferative effect was detected on NHDF cells at the IC_{50} concentrations determined in PANC-1, all three molecules reduced the viability of NHDF cells at least an order-of-magnitude higher concentrations.

4.1.2. TBP-134 arrests the cell cycle of PANC-1 cells in the G2/M phase

After 48 hours of treatment with 0.5 μM TBP-134, a G2/M phase arrest was detected, which can be seen as a significant increase in the relative cell numbers in the G2/M phase, coupled with a significant reduction in the G1 phase. G1 phase arrest was detected after treatment with 0.5 μM TBP-135, as the proportion of G1-phase cells increased. However, ONC201 did not affect the cell cycle at this time and concentration. After 72 hours of treatment with TBP-134, similar tendencies were observed, with a slightly enhanced effect. While TBP-135 and ONC201 influenced the cell cycle similarly after 72 hours, promoting the G1 phase arrest. Interestingly, the proportion of the sub-G1 phase, which indicates the population of apoptotic bodies with DNA fragments formed during apoptosis, did not increase significantly in either case.

4.1.3. ONC201 and its analogues induce apoptosis

After 24 hours of treatment, TBP-134 at 10 and 25 μM increased the ratio of early apoptotic cells from 6.4% (DMSO control) to 10.1% and 11.0%, respectively. However, this effect was the most pronounced in the case of 72 hours of treatment, where 0.5 μM TBP-134 and TBP-135 could significantly increase the ratio of early apoptotic cells (DMSO control: 21.1%) to 43.8% and 53.5%, respectively. In contrast, even after 72 hours exposure to ONC201 did not induce apoptosis at the lowest concentration.

The morphometric changes were also monitored to examine the characteristic morphology typical of apoptosis. Also, this experiment detected significant differences only after 72 hours of treatment. The average cell area significantly decreased after treatment with 0.5 μM TBP-135 to 338.5 μm^2 (DMSO: 630 μm^2). While TBP-134 at a higher concentration (25 μM) could exert the same effect, decreased the area to 376 μm^2. Both analogues increased the optical thickness at 0.5 μM concentration, TBP-134 increased the parameter to 6.5 μm (DMSO: 1.45 μm) and TBP-135 to 5.9 μm. At 0.5 μM, the optical volume of the cells was affected only by TBP-134 treatment, it increased the volume to 3112.3 μm^3 (DMSO: 932.5 μm^3). Meanwhile, ONC201 had no influence on cell morphology at 0.5 μM.

4.1.4. The molecular mechanisms underlying the induced apoptosis

Four target genes (death receptors (*DR4, DR5*), *p53* and *TRAIL*) were chosen to measure the changes in their expression at the RNA level. The expression of *DR4* and *DR5* was increased the most after 24-hour treatment with TBP-135, showing a 3.7-fold and 5.9-fold upregulation, respectively. TBP-134 treatment for 48 hours led to the highest increase in *p53* expression, by 3.7-fold. Contrary to the literature, ONC201 treatment did not increase the expression of

TRAIL at any time point, similar results were seen after TBP-134 treatment. In contrast, TBP-135 treatment caused significant induction in the expression of *TRAIL* after 3 hours, with a 2.4-fold increase.

The protein profile analysis was carried out after a 72-hour treatment with the molecules at 0.5 μM on PANC-1 cells. Out of the 35 tested apoptotic proteins, the relative level of 16 proteins changed significantly (increased or decreased) compared to the DMSO-treated control. Among the 16 proteins, both pro-apoptotic and anti-apoptotic proteins were present. ONC201 treatment increased the levels of cleaved caspase-3, FADD, DR5, SMAC and phospho-p53 (S15, S46, S392), while treatment with TBP-135 elevated the levels of DR4, DR5, cleaved and pro-caspase-3, SMAC and phospho-p53 (S15, S46). However, after TBP-134 treatment, the levels of DR4 and HSP60 were increased, but the levels of several proteins decreased, like pro-caspase-3, FADD and phospho-p53 (S15, S46, S392). The anti-apoptotic protein levels were also influenced following ONC201 treatment, the levels of cIAP-1, HSP60 and XIAP were increased. Whereas after treatment with the fluorinated analogues, several proteins were undetectable, or their levels were decreased.

4.1.5. The synergistic effect of ONC201 in combination with bortezomib on A2058 melanoma cells

We have previously determined the synergistic effect of ONC201 and bortezomib on A2058 melanoma cells. We've seen that ONC201 did not have an IC_{50} value on the cell line; however, combined with bortezomib it reduced the effective concentration of bortezomib by approximately half from 23.1 nM to 13.5 nM, as shown in Angéla Takács' thesis. In this current thesis, the possible molecular background of the synergism was revealed. After 72 hours of treatment with ONC201 alone and in combination with bortezomib could

increase the expression of DR5 at the protein level in A2058 cells, however, at the same time, the qPCR analysis at the mRNA level showed different tendencies. The molecules, either alone or in combination, caused a decrease in the expression of DR5 at the RNA level after 72 hours of treatment. However, bortezomib alone and in combination with ONC201 (13.5 μM, 40.5 μM) increased the expression of DR4 by 1.2-fold, 1.4-fold and 1.6-fold, respectively.

4.2. The effects of daunorubicin-containing peptide conjugates on PANC-1 cells

4.2.1. GSSEQLYL sequence can successfully target PANC-1 cells

The first step of this study was to select the peptide sequence with the best targeting properties. For this, three sequences were chosen from a paper by Bedi et al., these were GSSEQLYL found in ConjA, ETPPSWGG in ConjB and EPSQSWSM(O) in ConjC. Of the three, only ConjA with the GSSEQLYL targeting peptide showed a viability-reducing effect on PANC-1 cells, it reduced the viability to 8.6% at 10 μM, so it was selected as the parent sequence. Next, the position of the amino acid, which can be modified without the loss of the viability-reducing effect, was determined with the help of the so-called Ala-scan. The result of this experiment was that in position 6 the amino acid can be changed without the loss of effect, which resulted in Conj03 (targeting sequence: GSSEQAYL) with the best viability decreasing effect (14.9%). In position 6, further screening was carried out with numerous coding and non-coding amino acids and halogenated amino acid derivates. After the substitution of Leu^6 with para-chloro-phenylalanine (Conj16 with targeting sequence: GSSEQF(pCl)YL), an anti-tumour effect similar to that of the parent sequence was observed (9.7%).

Direct cellular cytotoxicity was assessed after 48- and 72-hours of treatment

with the conjugates (ConjA, Conj03 and Conj16) and Dau. After 48 hours, neither conjugates caused direct cytotoxicity at 10 μM. In contrast, at 100 μM they all induced a significant direct cytotoxic effect, which was further amplified after 72 hours. At 72 hours, Conj16 at 100 μM (51.9%) nearly achieved the same cytotoxic effect as 10 μM Dau (55.3%). At a concentration of 10 μM, the cytotoxicity % resulting from ConjA (19.8%) and Conj03 (16.0%) treatments remained comparable to the medium control (16.3%), whereas Conj16 increased direct cellular cytotoxicity slightly to 24.3%.

4.2.2. Chlorination enhanced the uptake of the conjugates in PANC-1 cells

A primary observation was made that the geometric mean values of the cellular fluorescent intensity for the conjugates were significantly lower compared to the Dau. This discrepancy in relative fluorescence intensity may be attributed to the conjugation process, as the fluorescence signal of free Dau can decrease upon conjugation with peptides. Despite this reduction, the cells effectively internalised the conjugates. We hypothesise that the uptake of the conjugates occurs via receptor-mediated endocytosis, whereas Dau enters cells through passive diffusion or the ’flip-flop’ mechanism. Among the three conjugates tested, the cellular uptake of Conj16 exhibited the highest level.

4.2.3. Conj16 induced apoptosis after 48-hour treatment

After 48 hours, Conj16 increased the ratio of late apoptotic cells (AnnexinV positive and To-Pro 3 positive) from 11% to 20%. After 72 hours, Conj16 increased the ratio of early apoptotic cells (AnnexinV positive and To-Pro 3 negative) from 1.7% to 9.3% and late apoptotic cells from 5.3% to 41.7% compared to the medium control. Furthermore, the induction of late apoptosis by Conj16 was comparable to Dau. In contrast, ConjA and Conj03 did not

exhibit significant apoptotic effects at either 48 or 72 hours.

4.2.4. Conj16 treatment influences the morphology of the cells, showing the characteristic features of cellular senescence

After 24 hours of treatment with 10 μM Conj16, the morphology of the cells had already significantly changed, which can be seen on the holographic images of the cells. After 24 hours of treatment with Conj16, the average cell area and optical volume increased, while the optical thickness remained unchanged. After 48 hours, the first two parameters further increased, and the optical thickness was also affected. However, the most pronounced effect was observed after 72 hours of treatment, resulting in a significant increase in all three parameters monitored: the cell area increased to 2168.8 μm^2 (Medium: 577.7 μm^2), the optical volume increased to 5963.1 μm^3 (Medium: 1001.1 μm^3), and the optical thickness increased to 3.0 μm (Medium: 1.6 μm). In contrast, ConjA and Conj03 did not significantly alter cell morphology even after 72 hours. These results indicate Conj16 appears to induce cellular senescence within 24 hours, whereas the reference molecule Dau induces apoptosis as early as 48 hours, as reflected in the holographic microscopy images.

4.2.5. The molecular background of the cellular senescence induced by Conj16

First, changes in the expression of 88 senescence-related genes were screened and the expression of 24 genes was upregulated. Based on these findings, 14 genes, mainly involved in cell cycle regulation, were selected to compare the senescence-inducing effects of the three conjugates. After 48 hours of treatment with 10 μM Conj16, the expression of *CCNE1, CDC25A, CDKN1A, E2F1, E2F2*, and *TERT* was significantly upregulated by more than 2-fold, whereas *CCNA1* expression was downregulated by 0.18-fold. Following treatment with ConjA and Conj03, the expression of *CCNA1, CCNE1,*

*CDC25A, CDC25*C, and *E2F3* was similarly affected but to varying extents compared to Conj16. Differing to Conj16, ConjA treatment did not influence expression of *CDKN1A, E2F1, E2F2, GLB1, LGALS3* and *TERT*.In contrast, Conj03 treatment did not affect the expression of *CDKN1A, E2F1, E2F2, GLB1*, and *LGALS3* but downregulated *CCND2* by 0.5-fold.

4.2.6. Treatment with all three conjugates at 10 µM showed no cardiotoxic effects compared to free Dau.

Treatment with the highest concentration of Conj16 (100 µM) and 10 µM Dau reduced cell viability to 3%, while treatment with 100 µM ConjA and Conj03 resulted in cell viability of over 60%. However, after treatment with all three conjugates at 10 µM the viability of HL-1 cells remained over 80%. Notably, 10 µM ConjA decreased viability to a greater extent than 10 µM Conj16.

5. Conclusions

This thesis has shown that the fluorinated ONC201 analogues exert a superior anti-tumour effect compared to ONC201, and it also highlights the tumour-targeting and anti-tumour effects of daunorubicin-based peptide conjugates on PANC-1 cells.

- ONC201 and its meta- and para-fluorinated analogue exert potent anti-tumour effects on PANC-1 cells with IC_{50} values ranging from 0.35-6.1 μM.
  a) The analogues not only act as potent anti-tumour agents on the PANC-1 cells, but they also spare the healthy fibroblast cells.
  b) They did not cause direct cytotoxicity after 72 hours of treatment at 0.5 μM.
  c) TBP-134 at 0.5 μM caused G2/M phase arrest in the cell cylce of PANC-1 cells after 48-hour treatment, while TBP-135 halted the cell cycle in G1 phase.
  d) Both fluorinated analogues successfully induced apoptosis after 72 hours of treatment at 0.5 μM, as evidenced by the holographic images as well.
  e) TBP-135 induced the expression of TRAIL at the RNA level after 3 hours of treatment, while ONC201 and TBP-134 did not affect the expression.
  f) TBP-135 activated both the extrinsic and intrinsic pathways similarly to ONC201, while TBP-134 predominantly activated the intrinsic pathway.
  g) ONC201, in combination with bortezomib, could significantly increase the expression of *DR4* at the RNA level in metastatic melanoma A2058 cells.

- The daunorubicin-based peptide conjugates can successfully deliver Dau to PANC-1 cells and deploy the anti-tumour effect of the drug.
  h) The sequence found in ConjA (GSSEQLYL) had the best effect on lowering the viability of PANC-1 cells.
  i) The amino acid in the targeting sequence at position 6 can be freely changed without losing the anti-tumour effect, resulting in Conj03.
  j) Conj16, with para-chlorinated phenylalanine found in position 6, showed the most potent anti-tumour effect out of 18 screened PDCs.
  k) After 48 hours of treatment at 10 μM, neither conjugate caused direct cytotoxicity to PANC-1 cells.
  l) Conj16 was most efficiently taken up by the cancer cells, showing signs of senescence at both the cell morphological and molecular levels, characterised by increased *CDKN1A* and decreased *CCNA1* mRNA levels, and inducing subsequent apoptotic cell death.
  m) The conjugates exhibited no cardiotoxicity at 10 μM, whereas Dau induced it severely.

6. Bibliography of candidate's publications

6.1. List of publications used for the thesis

1. **Zsófia Szász**, Kata Nóra Enyedi, Angéla Takács, Nóra Fekete, Gábor Mező, László Kőhidai, Eszter Lajkó (2024) Characterisation of the cell and molecular biological effect of peptide-based daunorubicin conjugates developed for targeting pancreatic adenocarcinoma (PANC-1) cell line. Biomedicine & Pharmacotherapy. 173:116293

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The cumulative impact factor: 38.942

Number of Q1 publications: 4

Number of D1 publications: 3