SELENOCOMPOUNDS AS NOVEL ANTICANCER AGENTS AGAINST RESISTANT BREAST CANCER AND ANTIBACTERIAL AGENTS IN *CHLAMYDIA TRACHOMATIS* D

Ph.D. Thesis

Andrea Csonka, M.D.

Supervisor: Gabriella Spengler, Ph.D.

Department of Medical Microbiology
Albert Szent-Györgyi Medical School
University of Szeged

Szeged

2022

1. INTRODUCTION

Among the EU member states, the incidence of lung and colon cancers and the mortality associated with these tumors is the highest in Hungary. Breast cancer is an important health problem with an increasing global trend of prevalence and mortality rate. It is the most frequently diagnosed neoplastic disease in women and one of the most important causes of death among them. The goal of chemotherapy is the elimination or reduction of malignant cell mass and to improve the quality of life of the patient. The use of chemotherapy could be complicated due to its low bioavailability, disadvantageous side effects due to non-selective cytotoxic activity and the emergence of multidrug resistance, whereby tumors show resistance to chemotherapeutic agents of various structures and mechanisms of action.

Chlamydiae are Gram-negative, obligate intracellular pathogens and symbionts of diverse organisms, ranging from humans to amoebae. *Chlamydia trachomatis* can cause a wide spectrum of human infections such as genitourinary infections in both gender and ocular infections, like neonatal conjunctivitis and trachoma. According to the WHO, the estimated number of new chlamydial infections were 129 million in 2020. Recurrent or persistent infection occurs in 10%-15% of women who are treated for *C. trachomatis* infection.

Drug resistance is a generally-known and widespread fact that develops when diseases become resistant to medical treatment. The most important factor of bacterial and cancer drug resistance is assigned to the MDR efflux transporter proteins removing toxic substances and chemotherapeutics out of the cells. Considerable number of compounds have been described with the ability to inhibit the function of the efflux pumps. An emerging therapeutic strategy is the use of chemosensitizers as adjuvants reversing the MDR phenotype.

Selenium (Se) is a trace non-metal element, but it is sometimes considered a metalloid. It is present in small amounts in the human body, but it is of essential importance to human biology and health. Se compounds have chemopreventive, antiproliferative and cytotoxic activities against cancer, as well as antioxidant or prooxidant activity, modulating inflammatory process, apoptosis induction, inhibition of multidrug efflux pump as P-gp, inhibition of the cancer metastasis. Se-compounds also possess antiviral, antimicrobial, anti-biofilm and antifugal properties. Based on literature data selenium-containing compounds could provide new alternatives in experimental chemotherapy to overcome multidrug resistance in cancer and bacteria.

2. AIMS OF THE STUDY

The aim of our study was to investigate the activity of eleven (**1-11**) selenocompounds on MCF-7 breast cancer cell line and its doxorubicin resistant subline KCR, as *in vitro* model system. Their potency in combination with doxorubicin was studied on MCF-7 and KCR breast cancer cell lines;furthermore, their activity as apoptosis inducers was studied in both breast cancer cell lines. The antibacterial effects of selenocompounds were evaluated in *C. trachomatis* D.

The main goals of the study were the following:

1) Determination of the cytotoxic effects of selenocompounds on doxorubicin sensitive MCF-7 and doxorubicin resistant KCR breast cancer cell lines by MTT assay.

2) Characterization of the activity of selenocompounds in combination with doxorubicin on MCF-7 and KCR breast cancer cells by checkerboard assay.

3) Evaluation of the apoptosis inducing effect of selenocompounds on MCF-7 and KCR breast cancer cell lines by Annexin V-FITC and propidium iodide double staining by using flow cytometry.

4) Determination of antibacterial activity of selenocompounds on *C. trachomatis* D by indirect immunofluorescence assay.

3. MATERIALS AND METHODS

3.1. Compounds studied

The eleven selenocompounds including cyclic selenoanhydride (**1**) and selenoesters (**2–11**) were kindly provided by Dr. Enrique Domínguez-Álvarez (Spanish National Research Council, Madrid, Spain) and by Prof. Dr. Carmen Sanmartín (University of Navarra, Pamplona, Spain). All compounds were stable, and their purity was assessed via spectroscopic techniques. Compounds (**12–15**) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), to be used as non-selenium (**12**) isostere of selenoanhydride (**1**) and inorganic chalcogen salts (**13–15**), for comparing their activity with the selenoesters. The stock solutions (in 10 mM concentration) of compounds were dissolved in dimethyl sulfoxide (DMSO).

3.2. Cell lines

Breast cancer cell line MCF-7 (ATCC® HTB-22) was purchased from LGC Promochem (Teddington, Middlesex, UK). The MCF-7 cell line and its drug-resistant subline KCR were grown in Eagle's minimal Essential medium (EMEM), containing 4.5 g/L glucose supplemented with a non-essential amino acid mixture, a selection of vitamins and 10% heat-inactivated fetal bovine serum (FBS). The cell lines were incubated at 37°C, in an atmosphere of 5% CO_2 and 95% air. On every third passage, 0.56 μg/mL doxorubicin was added to the medium in order to maintain ABCB1 expression in KCR cells.

3.3. Bacterial strain

C. trachomatis reference strain (serovar D, UW-3/Cx, ATCC, VR-885D) was used in the anti-chlamydial assay.

3.4. Cytotoxicity assay

The cytotoxic effects of the Se-compounds were determined on MCF-7 and KCR breast cancer cell lines. The effects of increasing concentrations of Se-compounds on cell growth were tested in 96-well flat-bottomed microtiter plates. The stock solutions (10 mM) of the compounds were diluted in 100 μL of EMEM medium.

The adherent breast cancer cell lines were cultured in 96-well flat-bottomed microtiter plates, by using EMEM supplemented with 10% heat-inactivated FBS. The density of the cells was adjusted to 1×10^4 cells in 100 μL/well, the cells were seeded for 24 h at 37°C, with 5% CO_2 prior to the assay, then the medium was removed from the plates containing the cells, and dilutions of Se-compounds previously made in a separate plate were added to the cells in 200 μL. The culture plates were incubated at 37°C for 24 h; at the end of the incubation period, 20 μL of MTT solution was added to each well. After incubation at 37°C for 4 h, 100 μL of 10% SDS solution was added to each well and the plates were further incubated at 37°C overnight. Cell growth was determined by measuring the optical density (OD) at 540/630 nm with Multiscan EX ELISA reader (Thermo Labsystems, Cheshire, WA, USA). Results are expressed in terms of IC_{50}, defined as the inhibitory dose that reduced the growth of the cells exposed to the tested compounds by 50%.

The selectivity was calculated by using the selectivity index (SI), which is defined as the quotient of the IC_{50} value determined for the non-tumorous MRC-5 cell line described previously (Domínguez-Álvarez *et al. Bioorg Med Chem Lett.* **26**:2821-24, 2016) to the IC_{50} value for the respective cancer cell line (MCF-7 or KCR). Compound was found to be strongly selective when its SI was 6 or higher furthermore, compounds with SI values of 1-3 and 3-6 were regarded as slightly and moderately selective, respectively.

Regarding the anti-chlamydial activity, cytotoxicity assay was performed on HeLa cells by the use of the same method as described above, to evaluate the concentrations at which the Se-compounds exert no direct toxic effects to these cells.

3.5. Checkerboard combination assay

A checkerboard microplate method was applied to study the effect of drug interactions between the Se-compounds and the chemotherapeutic drug doxorubicin. The assay was carried out on MCF-7 and KCR breast cancer cell lines. The adherent breast cancer cell lines were cultured in 96-well flat-bottomed microtiter plates, by using EMEM supplemented with 10% heat-inactivated FBS. The density of the cells was adjusted to 6×10^3 cells in 100 μL per well, the cells were seeded for 24 h at 37°C with 5% CO_2 prior to the assay and then the medium was removed from the plates containing the cells.

The final concentration of the Se-compounds and doxorubicin used in the combination experiment was chosen in accordance with their cytotoxicity towards these cell lines. The dilutions of doxorubicin were made in a horizontal direction in 100 μL, and the dilutions of the Se-compounds vertically in the microtiter plate in 50 μL volume. The plates were incubated for 72 h at 37°C in 5% CO_2 atmosphere. The cell growth rate was determined after MTT staining. At the end of the incubation period, 20 μL of MTT solution was added to each well. After incubation at 37 °C for 4 h, 100 μL of 10% SDS solution was added to each well and the plates were further incubated at 37°C overnight. OD was measured at 540/630 nm with Multiscan EX ELISA reader. Combination index (CI) values at 50% of the growth inhibition dose (ED_{50}) were determined by using the CompuSyn software (www.combosyn.com, ComboSyn, Inc., Paramus, NJ. 07652, USA) to plot 4 or 5 data points at each ratio. CI values were calculated by means of the median-effect equation, according to the Chou-Talalay method, where $CI<1$, $CI=1$, and $CI>1$ represent synergism, an additive effect (or no interaction) and antagonism, respectively.

3.6. Apoptosis induction

The ability of Se-compounds to induce apoptosis was determined on breast cancer cell lines. The apoptosis assays were performed by using Annexin V-FITC Apoptosis Detection Kit from Calbiochem (EMD Biosciences, Inc. La Jolla, CA, USA), following the instructions provided by the manufacturer. This assay enables the quantification of early and late apoptotic events, as well as necrosis and cell death in the cell population exposed to the Se-compounds. The density of the cell suspension was adjusted to 1×10^6 cells/mL. The cell suspension was distributed into 0.5 mL aliquots (5×10^5 cells) to a 24-well microplate and incubated overnight at 37°C in 5% CO_2. On the following day, the medium was removed, and fresh medium was added to the cells. The cells were then incubated in the presence of compounds at 1 or 2 μM for 3 h at 37°C. 12*H*-benzo[α]phenothiazine (M627), which is a known early apoptosis inducer, was used as a positive control. The samples were washed in phosphate buffered saline and fresh EMEM medium was added to the cells, followed by the incubation of the plate for 24 h at 37°C, in 5% CO_2. After the incubation period, the cells were trypsinized. The harvested cells were centrifuged at 2000×g for 2 minutes. The cells were then re-suspended in fresh serum-free EMEM medium. Thereafter, the apoptosis assay was carried out according to the rapid protocol of the kit and the fluorescence was analyzed immediately using a Partec CyFlow flow cytometer (Partec, Münster, Germany).

3.7. Propagation of Chlamydia trachomatis D

C. trachomatis D was propagated on HeLa 229 cells (ATCC, CCL-2.1). The titer of the infectious elementary bodies was determined by indirect immunofluorescence assay.

Serial dilutions of the elementary bodies' preparation were inoculated onto HeLa monolayers and after a 48 h culture, cells were fixed with acetone and stained with monoclonal anti-Chlamydia LPS antibody (AbD Serotec, Oxford, UK) and FITC-labelled anti-mouse IgG (Merck KGaA, Darmstadt, Germany). The inclusions of *C. trachomatis* D were enumerated under a UV microscope.

3.8. Anti-chlamydial assay

Elementary bodies of *C. trachomatis* D (4×10^3 IFU/mL) were incubated with compounds at selected concentrations (0.25 μM, 0.5 μM, 1.25 μM, and 2.5 μM) in sucrose-phosphate-glutamic acid buffer (SPG) for 2 h at 37°C.

As a control, *C. trachomatis* D was also incubated in SPG alone. To quantify the anti-chlamydial effects of compounds, HeLa cells were seeded in 24-well plates with 13 mm cover glasses. The confluent cells were infected with Se-compounds-treated *C. trachomatis* D or the non-treated controls. After 48 h, the cells were fixed with acetone at - 20°C for 10 min. The titer of the infectious elementary bodies was determined by indirect immunofluorescence assay.

4. RESULTS

4.1. Cytotoxicity assay

Eleven selenocompounds (**1–11**) and four reference compounds (**12**–**15**) were evaluated for their anticancer activity against MCF-7 and KCR breast cancer cell lines. The screening of the anticancer activity of Se-compounds in MCF-7 cells indicated that selenoanhydride (**1**) and selenoesters (**2–7**) were not cytotoxic towards this cell line, as all the IC_{50} values of these derivatives were above 100 μM. In contrast, ketone-containing selenoesters (**9–11**) showed a potent low-micromolar activity, as their IC_{50} values ranged from 1.04 to 1.70 μM, whereas the IC_{50} of phenoxycarbonylmethyl selenoester (**7**) was 64.8 μM. The results were similar for the resistant KCR cells except for two derivatives. First, in this case the IC_{50} of selenoanhydride (**l**) was at 2.35 μM, which was more than 40-fold lower than that of MCF-7, suggesting that this compound acts directly on ABCB1 overexpressed by KCR cells. Second, compound (**11**) was close to 2-fold less active on KCR cells compared to MCF-7. None of the compounds (**12–15**) evaluated for comparison studies exerted cytotoxic effects at concentrations below 100 μM on any of KCR, MCF-7 and MRC-5 cell lines evaluated. The anticancer effect of Se-compounds on MRC-5 human embryonic lung fibroblast cell line was determined previously . (Domínguez-Álvarez *et al. Bioorg Med Chem Lett.* **26**:2821-24, 2016)

Regarding the selectivity of the selenoesters towards cancer cells, it was clearly observed that ketone-containing selenoesters exerted a moderate selectivity towards MCF-7 and KCR cancer cells with respect to the non-tumorous MRC-5 lung fibroblast cells, with the exception of compound (**11**) which was slightly selective towards KCR, exhibiting a SI of 2.2. The SI of compound (**9**) for KCR cells was close to 6 (SI=5.6), which was the threshold for considering that a compound is strongly selective.

The remaining selenoesters lacked selectivity due to their poor activity against MCF-7 and KCR. In contrast, selenoanhydride (**1**) was strongly selective towards KCR cells in comparison to the non-tumorous fibroblast cells with a SI of 42.

4.2. Combination assay

The five active compounds (**1, 8-11**) in the cytotoxicity assay were evaluated in combination with doxorubicin on KCR and MCF-7 cells. The results were quite convincing as showed a marked difference between the two tested cell lines. All Se-compounds assayed exerted synergistic interactions with doxorubicin in KCR cell line, whereas all the observed interactions of the selenoesters with doxorubicin in MCF-7 cell line were antagonistic.

Against KCR cells, compound (**9**) was undoubtedly the most beneficial in the combination assay, as it exerted the highest grade of synergy among all evaluated compounds and at the lowest concentrations of both Se-compound (2.5 μM) and doxorubicin (42.5 μM). The remaining Se-compounds interacted in a synergistic manner with doxorubicin at a concentration of compound and drug 4- and 2-fold higher, respectively. Against MCF-7 cells, compound (**9**) interacted in a moderately antagonistic manner at a higher concentration (5 μM). Slight antagonism was observed for compound (**8**) at the same concentration, but the concentration of doxorubicin was in this case 4 times as high.

4.3. Apoptosis assay

Se-compounds were not able to induce significant apoptotic events in MCF-7 and KCR cells; with the exception of the selenoester (**7**) in MCF-7 cells. This derivative, at a low concentration (2 μM), triggered early apoptotic and late apoptotic/necrotic events in 16.9% and 7.85% of cells, respectively. This apoptosis-inducing activity was moderate, as reference compound M627 induced early apoptosis in 20.8% and late apoptosis in 67.1% of the population.

4.4. Anti-chlamydial assay

Before the assessment of the anti-chlamydial activity of the Se-compounds, a cytotoxicity assay was performed on HeLa cells to determine the ranges of concentrations at which the Se-compounds can be evaluated without showing direct toxic effects to HeLa cells.

Se-compounds (**2**, **3**, **5**, **7**)**,** and (**9-11**) significantly inhibited the formation of chlamydial inclusions at selected concentrations. Compounds (**2**) and (**7**) at 2.5 μM showed 82% and 71% inhibition, compared to the control, respectively. In addition, (**2**) and (**7**) were effective at 1.25 μM, whereas (**9**) and (**10**) inhibited the formation of inclusions at low submicromolar concentrations of 0.5 μM. The most potent anti-chlamydial Se-compounds were (**9**) and (**11**), as they inhibited more than 50% the growth of *C. trachomatis* D at the concentration of 0.25 μM (0.0689 μg/mL and 0.0858 μg/mL, respectively).

5. DISCUSSION

Conventional chemotherapy in the treatment of early and metastatic breast cancer is partly based on the administration of anthracycline drugs *e.g.* doxorubicin. Since these drugs provoke side-effects such as cardiotoxicity and myelosuppression, there is an urgent need to minimize the side-effects. In order to reduce the adverse effect of anthracyclines, several alternatives could be applied, for example, the use of liposomal doxorubicin, nanotechnology, and the development of less toxic derivatives.

In this study, we investigated the antitumor and cytotoxic properties of Se-compounds and their interaction with doxorubicin in order to find effective adjuvants for combination chemotherapy.

Chlamydia species can develop resistance for antibiotics, and this may show single drug or multidrug resistance due to several molecular mechanisms. Nowadays, only a few antibiotics, including tetracyclines, macrolides, and quinolone are in clinical use against these intracellular bacteria, because this species considered susceptible to antibiotics interfering with prokaryotic DNA-, RNA or protein synthesis. The development of new antibacterial and MDR reversing compounds is required to overcome this problem to find an effective therapeutic approach. The activity of Se-compounds against Chlamydia was investigated in order to find effective drugs that inhibit the reproduction of these intracellular bacteria.

5.1. Antitumor activity

As commented in the *Results* section Selenoanhydride (**1**) exerted selective activity towards the resistant KCR cell line overexpressing ABCB1 (IC_{50}=2.35 μM); however, it was ineffective against MCF-7 and MRC-5 (non-tumorous lung fibroblast) cells.

These results are in accordance with our previous data confirming that selenoanhydride (**1**) interacts directly with ABCB1.

Surprisingly, this derivative was unable to trigger apoptotic events in the tested breast cancer cell lines, probably due to a dual inhibition of ABCB1 and multidrug resistance protein 1 efflux pumps; nevertheless, other resistance mechanisms could also be involved.

Among selenoesters, only ketone-containing selenoesters (**9–11**) exerted significant cytotoxic activity against the breast cancer (KCR and MCF-7) cell lines. Symmetrical dimethyl selenodiesters (**2-5**) were inactive, as were amide-containing selenoester (**6**) and methoxycarbonylmethyl selenoester (**7**)**.**

In the latter, the replacement of the methyl moiety bound to the oxygen of the *O*-ester by a phenyl ring lowers the IC_{50} but still at a level between 60 and 100 μM. When this phenyl ester is replaced by a methylketone (**9**) or a *tert*-butylketone (**11**), the activity increases dramatically, this time lowering the IC_{50} to low micromolar concentrations, pointing to the crucial role of this alkylketone moiety in the biological activity of ketone-containing selenoesters. Furthermore, these promising selenium derivatives exerted a noteworthy selectivity towards the evaluated cancer cells (MCF-7, KCR) rather than the non-tumorous cell line MRC-5.

The results observed in combination assays are astonishing, in that they point to differential activity in the two cell lines, the resistant (KCR) one in this case being more sensitive to the action of the compounds. It has been shown that doxorubicin and methylseleninic acid act synergistically on MCF-7 cells, inducing apoptosis because doxorubicin and selenium cooperatively activate first apoptosis signal (FAS) pathway. Doxorubicin causes Fas oligomerization in a FasL-independent manner and methylseleninic acid increases FAS-associated death domain protein expression together triggering apoptosis. Out of our 11 Se-compounds, only methoxycarbonyl-methyl *p*-chlorobenzoselenoate (**7**) induced apoptosis in MCF-7 cells, and the other derivatives were not capable of provoking apoptosis of MCF-7 and KCR cells.

This is very relevant as it suggests that these derivatives might have the ability to overcome some aspects of resistance in KCR cells. Since the derivatives are proven ABCB1 modulators, their synergism with doxorubicin might be due to their interaction with this efflux pump overexpressed by KCR cells. On the contrary, the explanation of their antagonism with doxorubicin in MCF-7 cells is the involvement of other resistance mechanisms and cellular processes. This could open a new and straightforward approach to treat ABCB1-expressing resistant breast cancer that is resistant to the current treatments in clinical use. Methylketone

selenoester (**9**) would be in such cases the most promising compound. Its activity makes it worth to be investigated in more depth for potential applications and its closely related new derivatives, which could be synthesized in future work, with intrinsic anticancer activity as sensitizers of resistant cancer cells.

Overall, the results obtained herein highlight the importance for biological activity of the -$COSeCH_2COCH_3$ and -$COSeCH_2CO(CH_3)_3$ moieties in comparison with the remaining substituents considered (-$COSeCH_3$, -$COSeCH_2CONH_2$, -$COSeCH_2COOCH_3$, and -$COSeCH_2COPh$). The good cytotoxic activity, selectivity, and ability to modulate the effect of doxorubicin found for the ketone-containing selenoesters (**9–11**) against the two breast cancer cell lines evaluated are in agreement with the previous work of our group on mouse T-lymphoma cells (L5178Y) and colonic adenocarcinoma cells (Colo 205 és Colo 320) and it draws the attention to this privileged moiety. In future studies it will be necessary to obtain and evaluate more compounds with these moieties in order to ascertain what substituents in the phenyl ring bound to the carbonyl of the selenoester enhance activity, with the aim of designing more potent and selective anticancer agents.

5.2. Anti-chlamydial activity

Previous studies have reported that selected Se-compounds, such as certain selenocyanates, selenoureas, and diselenides, showed antiproliferative activities against the intracellular forms of *Leishmania* spp. Taking those results into account this study provided a new line of evidence for the action of selenoanhydride/selenoesters on an obligate intracellular chlamydial strain. In particular, different selenoesters, such as (**2**, **3**, **5**, **7**), and (**9–11**), have exerted a noteworthy activity against *C. trachomatis* D. Furthermore, the activities of the methyl (**9**) and the *tert*-butyl (**11**) derivatives were very promising, as they inhibited the formation of more than 50% of the chlamydial inclusions, at a very low concentration (0.25 μM). However, their mode of action has not been ascertained in this study.

Regarding the observed structure activity relationships of the anti-chlamydial assays, ketone selenoesters (**9–11**) showed noteworthy activity at lower concentrations (0.25 μM, and 0.5 μM), compared to the rest of the series (1.25 μM, and 2.5 μM). Among the remaining selenoesters, the symmetric dimethyl selenodiester, which contains thiophene ring (**2**), and the methyl oxoester derivative (**7**) showed a better activity, and the activities of the symmetric dimethyl selenodiesters (**3**) and (**5**) were also remarkable. This fact highlights the importance of the symmetry for the activity against intracellular pathogens.

Herein, we have reported the evaluation of the antitumor, multidrug resistance reversing and antibacterial activity of 11 novel Se-compounds. Selenoanhydride (**1**) exerted selective activity towards the doxorubicin-resistant KCR cell line overexpressing ABCB1.

Among the selenoesters, only the ketone-containing selenoesters promoted significant cytotoxic activity against MCF-7 and KCR cell lines, and Se-compounds acted synergistically with doxorubicin on the KCR cell line. Methylketone selenoester (**9**) showed potential activity against *C. trachomatis* D at very low concentration (0.25 μM). Based on the results, the importance of the $COSeCH_2COCH_3$ and $COSeCH_2CO(CH_3)_3$ moieties for the cytotoxic, adjuvant role and the anti-chlamydial effect of Se-compounds was highlighted. Furthermore, it can be concluded that this group of compounds can be attractive potential antitumor and anti-chlamydial lead scaffolds, for further development of new chemical tools, to overcome multidrug resistance.

6. NEW FINDINGS

1) Ketone-containing selenoesters (**9–11**) exerted significant cytotoxic activity against the doxorubicin sensitive MCF-7 and doxorubicin-resistant KCR breast cancer cell lines.

2) Ketone selenoesters (**9-11**) exerted a high or moderate selectivity towards the evaluated breast cancer cell lines (MCF-7, KCR) compared to non-tumorous MRC-5 cells.

3) Only methoxycarbonyl-methyl *p*-chlorobenzoselenoate (**7**) induced early and late apoptosis in MCF-7 cells.

4) Effective cytotoxic activity, selectivity, and synergistic activity with doxorubicin were found for ketone-containing selenoesters (**9–11)** against the doxorubicin-resistant KCR breast cancer cell line.

5) Different selenoesters, such as the methyl group selenoesters (**2**, **3**, **5**), methoxycarbonyl-methyl *p*-chlorobenzoselenoate (**7)**, and ketone-containing selenoesters (**9–11**), have exerted a noteworthy activity against *C. trachomatis* D.

6) Methylketone selenoester (**9**) showed the most potent antibacterial activity against *C. trachomatis* D at a very low concentration (0.25 μM).

7. ACKNOWLEDGEMENTS

I would like to express my deep and sincere gratitude to my supervisor, **Dr. Gabriella Spengler** for the opportunity to work in the *Experimental chemotherapy and prophylaxis* research group at the Department of Medical Microbiology. She raised my interest in the experimental work. Furthermore, I wish to thank her for the valuable comments, advice and scientific inspiration.

I would like to express my grateful thanks to **Prof. Dr. Katalin Burián** for the possibility to work at the Department of Medical Microbiology.

I am extremely grateful to our collaborator **Dr. Enrique Domínguez-Álvarez** for providing the selenocompounds, furthermore I am thankful for his professional advice during our work together.

I would like to thank **Dr. Imre Ocsovszki** for the help with the flow cytometry measurements.

I am grateful to my colleagues **Dr. Tímea Mosolygó and Dr. Annamária Kincses** for their friendship, support and kind words during my time in the laboratory. I owe a great deal of gratitude to **Anikó Vigyikánné Váradi**,for the excellent technical assistance in the laboratory. I express my thanks to all my co-workers, colleagues and staff members at the Department of Medical Microbiology, for creating a supportive and pleasant working environment.

I would like to acknowledge **Prof. Dr. József Molnár** for his support and help related to the research of bacterial and cancer multidrug resistance.

Finally, I feel a deep sense of gratitude to my **husband Ákos and my family** for their support.

8. FINANCIAL SUPPORT

The work on which this thesis was based was supported by the following organizations and grants:

- Foundation for Cancer Research Szeged (Szegedi Rákkutatásért Alapítvány)
- GINOP-2-3-2-15-2016-00038
- SZTE ÁOK-KKA 2018/270-62-2 of the University of Szeged, Faculty of Medicine, Hungary: Selenium derivatives as novel promising antimicrobial agents

9. PUBLICATIONS RELATED TO THE THESIS

I. **Csonka A**, Kincses A, Nové M, Vadas Z, Sanmartín C, Domínguez-Álvarez E, Spengler G. Selenoesters and Selenoanhydrides as Novel Agents Against Resistant Breast Cancer. Anticancer Res. 2019 Jul;39(7):3777-3783. doi: 10.21873/anticanres.13526. PMID: 31262904.

IF: 1.994

II. Mosolygó T, Kincses A, **Csonka A**, Tönki ÁS, Witek K, Sanmartín C, Marć MA, Handzlik J, Kieć-Kononowicz K, Domínguez-Álvarez E, Spengler G. Selenocompounds as Novel Antibacterial Agents and Bacterial Efflux Pump Inhibitors. Molecules. 2019 Apr 16;24(8):1487. doi: 10.3390/molecules24081487. PMID: 31014009; PMCID: PMC6514980.

IF: 3.267

Total IF: 5.261