Transition metal-catalysed transformations of skeletally modified estrone derivatives

Thesis of Ph.D. dissertation

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1. Introduction and aims

The goal of drug discovery is to develop drugs that are selectively active in the treatment of tumour diseases. Currently marketed drugs used in cancer therapy have limited applicability and usability due to a number of serious side effects. Although several estrone-based antitumor agents have been described in the literature as potential anticancer agent, however, their clinical use has been limited because of their strong estrogenic side effects. Inverting configuration of the C-13 carbon atom of the estrane scaffold abolishes the estrogenic effect. The resulting 13α-estrone derivatives exhibit significantly reduced affinity for estrogen receptors. Steroids produced in this way may serve as a basis for the development of estrone-based, selective, hormonally inactive anti-tumor compounds.

Based on this, the aim of my doctoral thesis was the synthesis of novel estrone derivatives with antiproliferative as well as non-hormonal activities. To eliminate the estrogenic effect, the13-epimer derivatives have selected as starting compounds. They were intended to convert into the corresponding substrates derivatized with functional groups having dual effects such as good leaving character and *ortho* activator.

1. Due to the presence of good leaving property of ester group, we designed the preparation of C-3 biphenyl derivatives using phenylboronic acid as reactant in the presence of $Ni(PCy_3)Cl_2$ catalyst via Suzuki–Miyaura cross-coupling reactions.
2. The second goal was to incorporate aryl groups at the C-2 position for the selected estrone precursors by means of palladium-based direct C–H activation exploiting the *ortho*-activation effect of the prepared phenol ester substrate.
3. Finally, we have plan a development of efficient synthetic route accessing potentially bioactive 13α-estrone based diaryl ethers by Chan–Lam coupling reactions.

Our additional effort focused on using more gentle conditions preferring microwave-assisted transformation techniques instead of traditional heating methods. After the reactions, the structure of the target products was determined using modern analytical methods and techniques (1D-NMR, MS). The antiproliferative effect of the newly synthesized compounds on human adherent cell lines of the female reproductive system and healthy fibroblast cell lines *in vitro* was studied in collaboration with the Institute of Pharmacodynamics and Biopharmacy, University of Szeged.

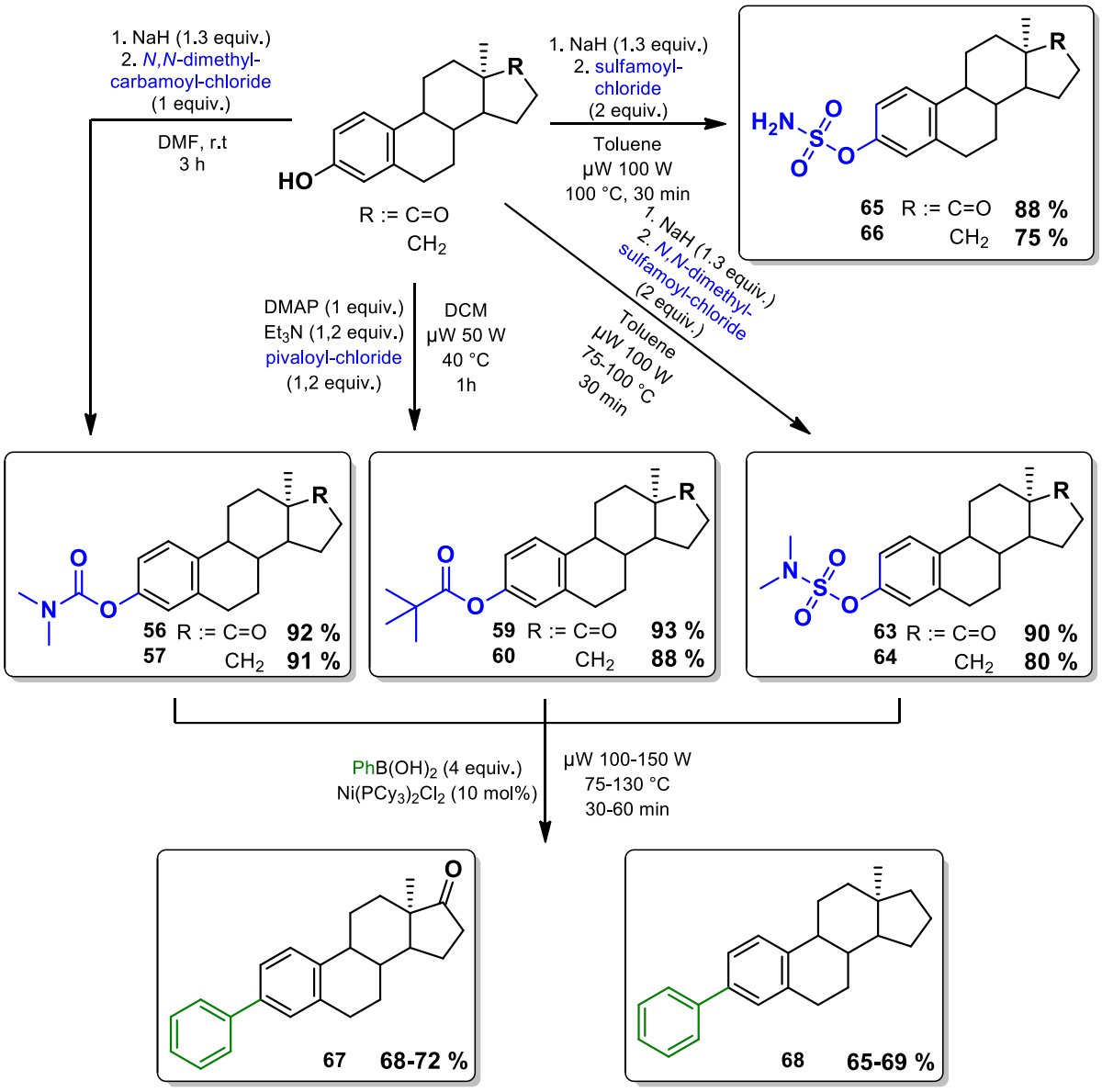
2. Applied methods

Most of the reactions were carried out on the millimolar scale and were monitored by thin-layer chromatography. For this Kieselgel 60 F254 (MERCK) sheets with a thickness of 0.2 mm were utilized. The palladium-catalysed C–H activations and Suzuki-Miyaura cross-couplings were carried out using microwave reactor (CEM Discover SP). The Chan–Lam coupling was performed at room temperature. Products were purified by column chromatography (silica gel 40–63 μm) or by simple filtration and/or recrystallization.

The new compounds were identified by means of one- and two-dimensional NMR spectroscopic techniques (Bruker DRX-500 spectrometer) and mass spectrometry (Finnigan TSQ-7000 triple quadrupole mass spectrometer). The commercially available reagents were purchased from Sigma-Aldrich, TCI Chemicals, Fluorochem and Molar and they were used without further purification. The biological assays were performed by the Institute of Pharmacodynamics and Biopharmacy, University of Szeged.

3. Novel scientific results

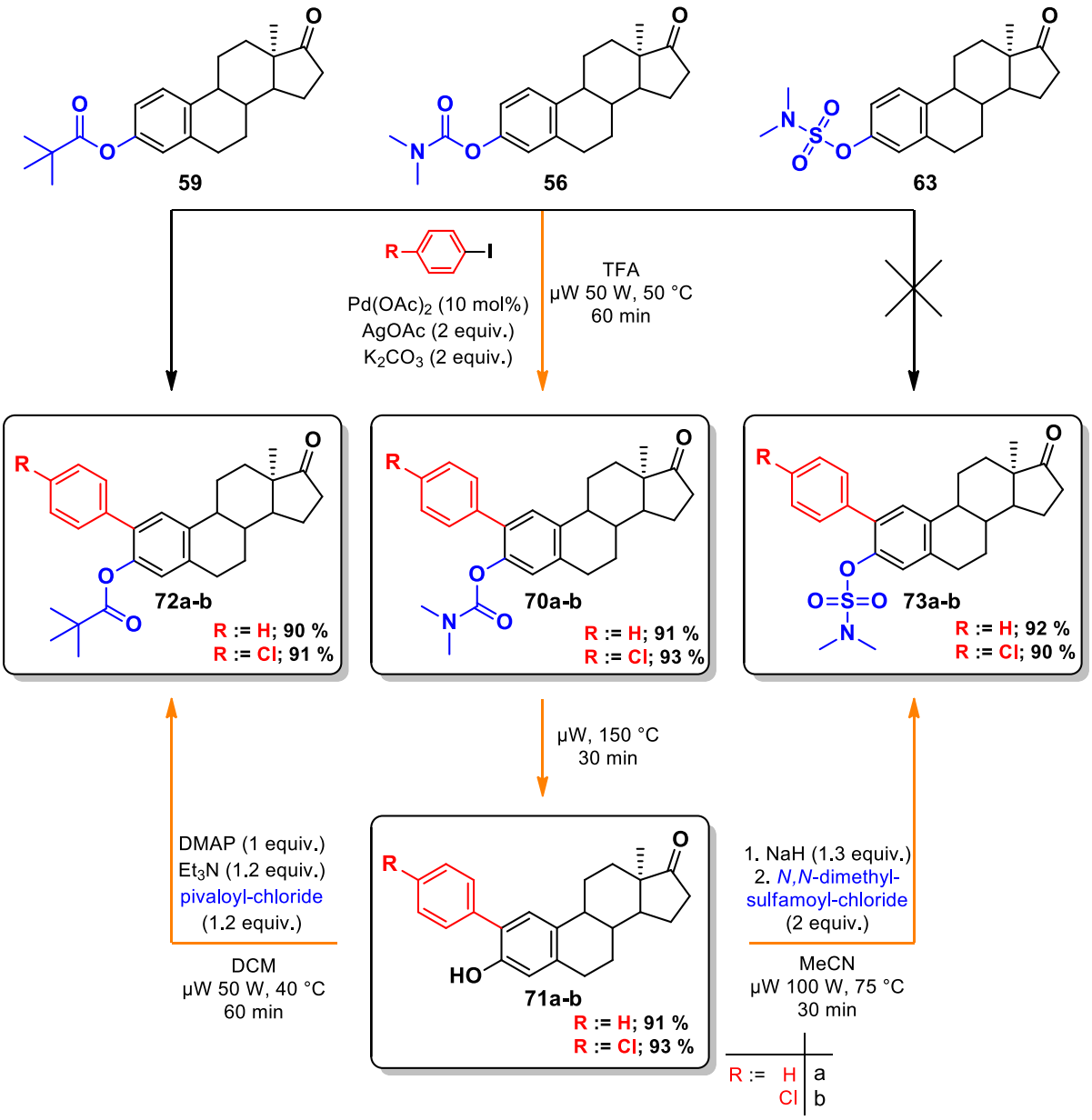
3.1. Biphenyl derivatives were prepared by Suzuki–Miyaura cross-coupling reaction starting from 3-hydroxy-13α-estra-1,3,5(10)-triene-17-one (4) and its 5 17-deoxy-based phenolic esters. The C-3 phenolic hydroxyl group of the A-ring of starting steroids was esterified by means of acylation and deprotonation steps. The desired **56**–**66** compounds were obtained in yields of 75–92%. Afterwards, they were exploited as substrates instead of aryl halides for the Suzuki–Miyaura cross-coupling treating with phenylboronic acids and in the presence of $Ni(PCy_3)_2Cl_2$ catalyst (**Scheme 1**). [1]



Scheme 1.

3.2. In order to improve the biological effect, the structure of the prepared 56, 59 and 63 phenol esters were modified by incorporating phenyl and *para*-chlorophenyl groups into the C-2 position utilizing palladium-catalyzed C–H activation. Since the synthetic method was not available for preparing of the **63** sulfamate, an alternative preparation route should be followed by selective cleavage of the carbamate group for **70** (in microwave reactor, 150 °C, 30 min.) then a sulfamoylation step of the observed **71a-b** 2-aryl-3-hydroxy derivatives. A noteworthy aspect of the reaction is that the earlier reported cleavage of leaving carbamoyl group required a prolonged refluxing of the reaction mixture in a NaOH-ethanol solution. In addition, the isolation needs a column chromatography purification. Following in our one-pot

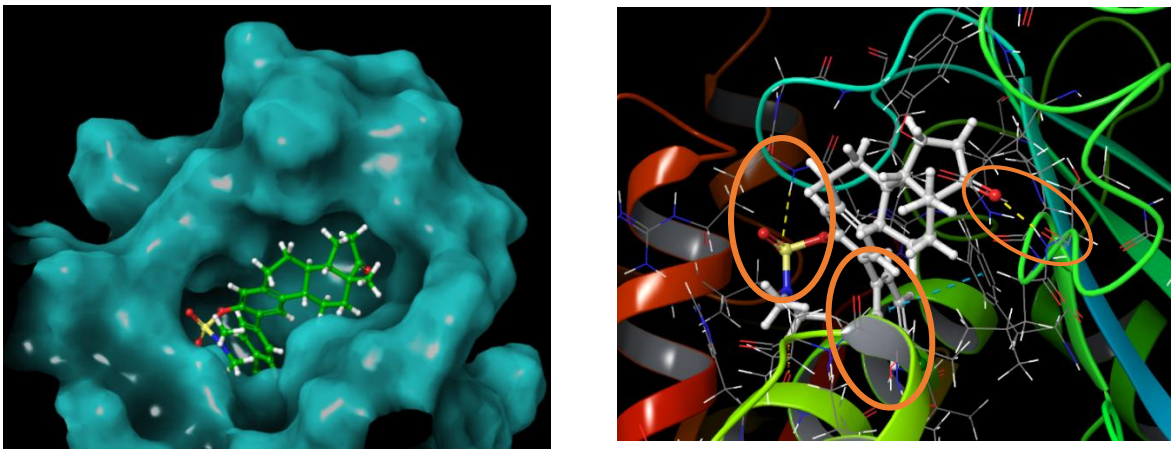
microwave assisted procedure, the whole synthetic procedure was accelerated and simplified (**Scheme 2**). [1]



Scheme 2.

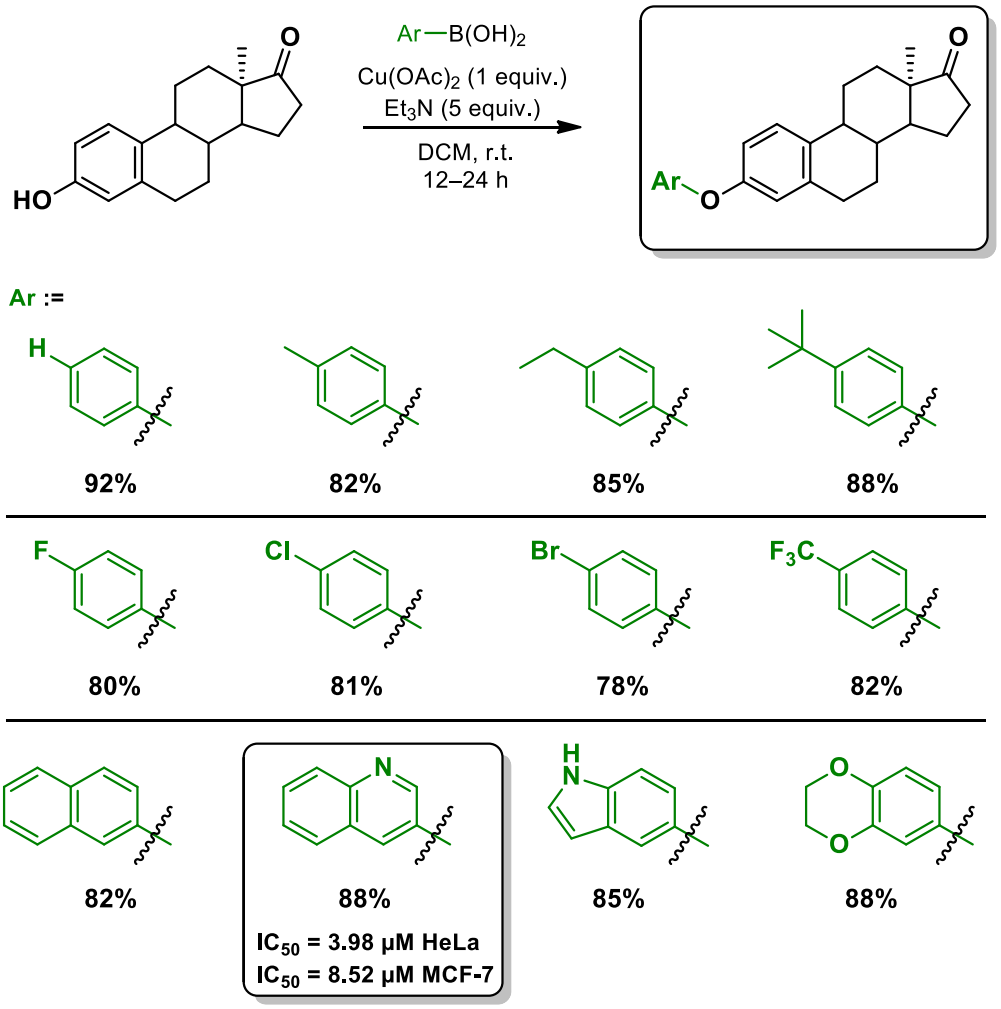
3.3. The molecular modelling investigated the affinity of 2-(*para*-chlorophenyl)-3-(*N*,*N*-dimethylsulfamoyloxy)-13α-estra-1,3,5(10)-triene-17-one (73b) for the taxol binding site of β-tubulin protein. The structure of the β-tubulin protein-taxol complex (PDB: 5SYF) was downloaded from the PDB database. The missing hydrogens, side chains, and loops were filled in. The relaxed tubulin protein the taxol binding site and the ligands were created followed by docking.

The molecular modelling demonstrated a strong correlation with biological activity. At the taxol binding site, the compound finds a hydrophobic pocket, the chlorophenyl moiety turns inward and the *N*,*N*-dimethylsulfamoyl group faces outward. In the hydrophobic environment, a π-π interaction forms with one of the phenylalanine residues of the protein. At the C-17 keto group an H-bridge interaction forms with the amino acid threonine of the protein. The presence of the keto group at C-17 position may justify the biological efficiency explaining its antimitotic effect (**Scheme 3**). [2]



Scheme 3.

3.4. A 12-membered chemical library was constructed starting from 3-hydroxy-13α-estra-1,3,5(10)-triene-17-one (4) precursor via Cu(II)-catalyzed Chan–Lam coupling (Yields: 78–92%). For this work, phenyl, substituted phenyl as well as heteroaryl boronic acids were applied (**Scheme 4**). Based on the literature background and our experimental results, we demonstrate a proposed reaction mechanism. [3]


Ar—B(OH)2
Cu(OAc)2 (1 equiv.)
Et3N (5 equiv.)
DCM, r.t.
12–24 h
HO
O
Ar
O
Ar :=
92%
82%
85%
88%
80%
81%
78%
82%
82%
88%
IC50 = 3.98 μM HeLa
IC50 = 8.52 μM MCF-7
85%
88%

Scheme 4.

4. Publications directly related to the dissertation (MTMT ID: 10069069)

1. **Traj P.**, Abdolkhaliq H. A., Németh A., Dajcs T. S., Tömösi F., Lanisnik-Rizner T., Zupkó I., Mernyák E. Transition metal-catalysed A-ring C–H activations and C(sp^2)–C(sp^2) couplings in the 13α-oestrone series and *in vitro* evaluation of antiproliferative properties; *J Enz Inhib Med Chem*, **2021**, 36, 895–902. DOI: 10.1080/14756366.2021.1900165.

IF: 5.756

2. Hazhmat A., **Traj P.**, Szebeni G. J., Gémes N., Resch V., Paragi G., Mernyák E., Minorics R., Zupkó I., Investigation of the Antineoplastic Effects of 2-(4-Chlorophenyl)-13-Estrone Sulfamate against the HPV16-Positive Human Invasive Cervical Carcinoma Cell Line SiHa; *Int. J. Mol. Sci.*, **2023**, 24, 6625–6641. DOI: 10.3390/ijms24076625.

IF: 5.600 (2022)

3. Kovács É., Hazhmat A., Minorics R., **Traj P.**, Resch V., Paragi G., Bruszel B., Zupkó I., Mernyák E., Synthesis and Antiproliferative Activity of Steroidal Diaryl Ethers; *Molecules*, **2023**, 28, 1196–1211. DOI:10.3390/molecules28031196.

IF: 4.600 (2022)

Total impact factor:15.956

5. Other publications

1. Madácsi R., **Traj P.**, Hackler L. Jr., Nagy L. I., Kari B., Puskás L. G., Kanizsai I. Synthesis and biological evaluation of 4,5,6,7-tetrahydrothieno [2,3-c]pyridine-based β-aminonitriles and their derivatives; β-amino carboxamides, (thio)ureas and tetracycles; *J Heterocyclic Chem* **2020**, *57*, 635-652. DOI: 10.1002/jhet.3800

IF: 1.484

2. Szabados M., Ádám A. A., **Traj P.**, Muráth Sz., Baán K., Bélteky P., Kónya Z., Kukovecz Á., Sipos P., Pálinkó I. Mechanochemical and wet chemical syntheses of CaIn-layered double hydroxide and its performance in a transesterification reaction compared to those of other $Ca_2M(III)$ hydrocalumites (M: Al, Sc, V, Cr, Fe, Ga) and Mg(II)-, Ni(II)-, Co(II)- or Zn(II)-based hydrotalcites; *J Cat*, **2020**, 391, 282–297. DOI: 10.1016/j.jcat.2020.07.038

IF: 7.760

Total impact factor: 9.244

6. Lectures and posters related to the dissertation

Lectures:

1. **Traj P.**, Németh A., Dajcs T. S., Mernyák E. Átmenetifém-katalizált keresztkapcsolási és C–H aktiválási reakciók a 13α-ösztron sorban; XLIII. Kémiai Előadói Napok, Szeged, **2020**.
2. **Traj P.**, Abdolkhaliqb H. A., Kovács É., Zupkó I., Mernyák E. Chan–Lam kapcsolások 13α-ösztron sorban; XLIV. Kémiai Előadói Napok, Szeged, **2021**.
3. **Traj P.**, Palládium-katalizált C–H aktiválási reakciók fenolon; MTA Szteroid- és Terpenoidkémiai Munkabizottsági Ülés, Szeged, **2021**.

Posters:

1. **Traj P.**, Abdolkhaliq H. A., Németh A., Dajcs T. S., Zupkó I., Mernyák E. Transition metal-catalyzed cross coupling and C–H activation reactions on 13α-estrone derivatives; 26th International Symposium on Analytical and Environmental Problems, Szeged, **2020**.
2. **Traj P.**, Mernyák E. Fenolok C–H aktiválási reakciói; XIV. Szent-Györgyi Albert konferencia, BME Budapest, **2021**.
3. Abdolkhaliq H. A., Mernyák E., **Traj P.**, Szebeni J. G., Minorics R., Zupkó I. Investigation of the anticancer potentials of the newly synthesized 13α-estrone derivatives; 11th ISCTICO-HUPHAR-IUPHAR Conference, Pécs, **2021**.
4. Resch V. E., **Traj P.**, Mernyák E., Paragi G. Computional study of modified steroids binding to microtubule; MTA Peptidkémiai Munkabizottság és MTA Kémiai Biológiai Munkabizottság Közös Tudományos Ülés, Balatonszemes, **2022**.