Modifications in ring A or D of estrane core

The theses of Ph.D. dissertation

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1. Introduction and aims

The majority of estrogen-dependent diseases might be treated by antihormonal drugs. Estrone-based drugs acting through inhibition of enzymes involved in estrogen biosynthesis usually have estrogenic activity, which is an undesirable side-effect. The development of drugs acting selectively is one of the major challenges in medicinal chemistry. Directed substitutions of ring A of estrone lead to compounds which may have substantial enzyme inhibitory effect against key enzymes in estrogen biosynthesis. Our aim was to synthesize selectively acting enzyme inhibitors based on hormonally inactive 13α-estrane core modified in ring A. We began our synthetic organic work with aromatic electrophilic substitutions furnishing halogenated 13α-estrone derivatives. The newly synthesized iodo and bromo derivatives proved to be suitable starting compounds for novel Pd-catalyzed C–C or C–N cross-coupling reactions in the 13-*epi*-estrone series. We have developed efficient microwave-assisted Sonogashira and Buchwald–Hartwig coupling procedures, which tolerated different functional groups present on ring A and allowed the formation of the desired coupled products even at sterically hindered positions. Our additional aim was to synthesize fluorescent-labelled estrone derivatives based on BODIPY dyes. The labelling was planned without the involvement of the main oxygen functionalities of estrone. Thus estrone-BODIPY conjugates labelled at C-2 or C-15 were synthesized, which may serve as good candidates for the development of imaging probes for biochemical assays.

2. Applied methods

Most reactions were carried out on a millimolar scale, and were monitored by thin-layer chromatography. CuAAC reactions were carried out by conventional heating and palladium-catalyzed cross couplings were performed in a CEM Discover SP microwave reactor. The purification of the reaction mixtures and the separation of the products were accomplished by flash chromatography. The structures of the new compounds were confirmed mainly by one- and two-dimensional NMR spectroscopic techniques.

3. Novel scientific results

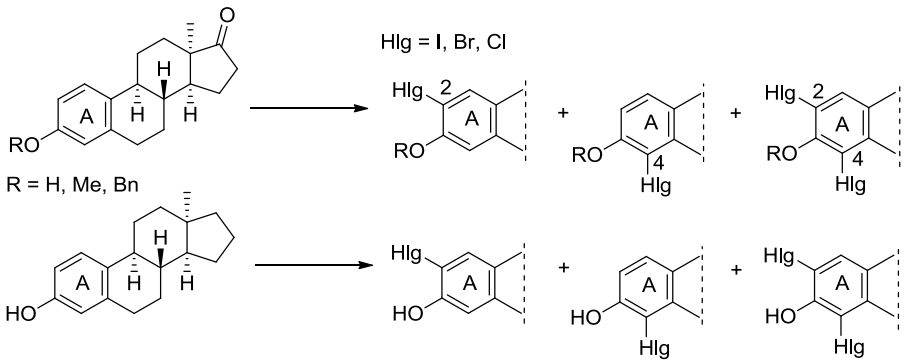
3.1. Introduction of halogens onto the aromatic ring of 13α-estrone with different protecting groups at position 3 (OH, OMe, OBn) was performed (Scheme 1). Chlorination, bromination or iodination was carried out with *N*-halosuccinimides (NXS) as electrophile triggers in different

solvents. In the 3-OH series both 2- and 4-substituted regioisomers were formed and 2,4-*bis*-substitutions also occurred. The reactions were completed in short reaction times and the mixtures of the desired products could efficiently be separated by flash chromatography.

3.2. The influence of the solvent applied and the quantity of reactant on product yields of the reactions discussed in 3.1. was investigated. It can be stated that conversions can be influenced by changing the solvent of the reaction. The most extreme example was the 2-bromo derivative of 13α-estrone, which formed only in dimethyl sulfoxide. The use of excess NXS in the reactions of 13α-estrone facilitates double substitution.

3.3. The impact of 3-methyl or 3-benzyl protecting groups on halogenations was investigated (Scheme 1). Substantial differences were found in product ratios. Monosubstitutions occurred in the 3-ether series, while halogenations of the phenolic starting compound led to both mono- and disubstituted derivatives. Iodination of the 3-benzyl ether could not be achieved via this method. Most reactions were carried out at room temperature. Chlorination of 3-ethers, however, required harsh conditions, namely, elevated temperature and the use of trifluoroacetic acid.

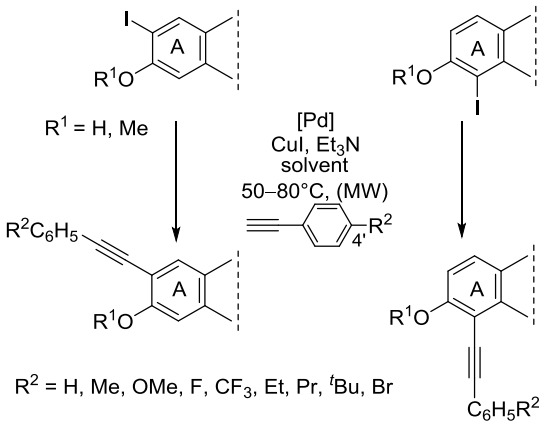
3.4. Halogenations of 17-deoxy-13α-estrone were carried out using *N*-halosuccinimides in different solvents furnishing 2-, 4- as well as 2,4-*bis*-substituted regioisomers (Scheme 1). We found that the presence or absence of the 17-keto function has great influence on the product ratio. A comparison of the results obtained in the halogenation of 17-oxo and 17-deoxy starting compounds allowed the conclusion that, depending on the nature of the C-17 substituent, different conditions are needed for the convenient synthesis of the desired mono- and disubstituted compounds. Chlorination of 17-deoxy-13α-estrone required the combination of reagent excess and microwave irradiation.



Scheme 1

3.5. A convenient synthetic microwave procedure was developed for the Sonogashira coupling of 2-iodo-3-*O*-methyl-13α-estrone (Scheme 2). Couplings at C-2 could efficiently be achieved using 0.1 equiv of $Pd(PPh_3)_4$ and CuI in dimethylformamide (DMF) as solvent in the presence of Et_3N as a base, at 50 °C for 20 min in a microwave reactor. After establishing the most favorable reaction conditions for phenylacetylene as alkyne partner, Sonogashira reactions were carried out with several *para*-substituted phenylacetylenes ($C_6H_5R^2$). All couplings resulted in the desired products in high yields, irrespective of the nature of the substituent at C-4'. The method was successfully adapted to compounds with C-3 phenolic OH by changing the solvent to acetonitrile.

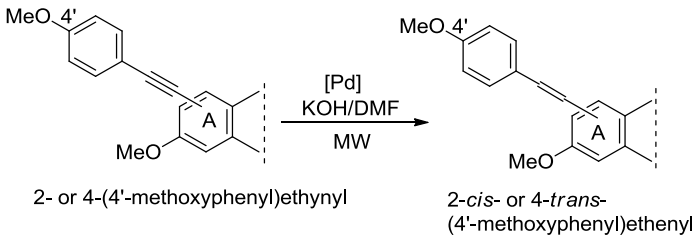
3.6. The method discussed in 3.5. was successfully adapted to coupling of 4-iodo-3-*O*-methyl-13α-estrone with phenylacetylene by minor changes in reaction conditions (Scheme 2). We found that elevation of the reaction temperature to 80 °C and the change of catalyst to $Pd(PPh_3)_2Cl_2$ facilitate substitution at C-4. The reaction of 4-iodo-13α-estrone bearing phenolic OH required the changing of solvent to tetrahydrofuran. We isolated the desired 4-phenethynyl-3-hydroxy derivative in excellent yield. The method was successfully adapted to the synthesis of 4-(4'-subst.)phenethynyl-13α-estrone derivatives bearing various 4'-substitutents.



Scheme 2

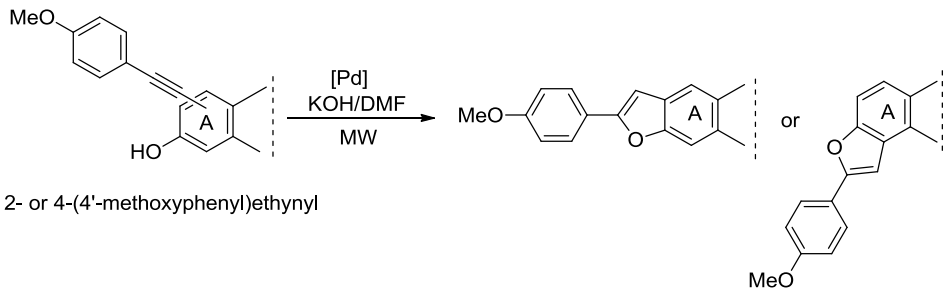
3.7. We performed the partial saturation of 3-*O*-methyl-4'-methoxy-13α-estrone derivatives by semihydrogenation using DMF/KOH as the hydrogen source and $Pd(PPh_3)_2Cl_2$ as the catalyst (Scheme 3). Full conversion of the starting compounds was achieved in 35 minutes by microwave irradiation. Both *cis*-alkenes and *trans*-alkenes were formed in chemo- and

stereoselective manner under the applied conditions from 2- and 4-phenethynyl derivatives, respectively. The *cis* or *trans* orientation of the resulting geometric isomers was deduced from vicinal coupling constants.



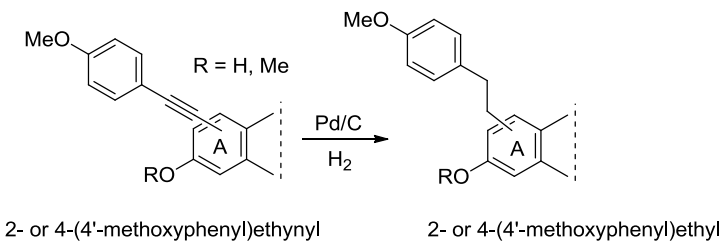
Scheme 3

3.8. Semihydrogenation of triple bonds of 3-hydroxy-4'-methoxy derivatives did not result in the desired 2- or 4- phenethenyl compounds by the method discussed in 3.7 (Scheme 4). Under the conditions used for partial saturation, the ethynyl derivatives bearing a phenolic OH group furnished benzo[b]furans.



Scheme 4

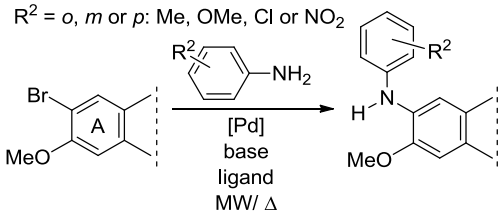
3.9. Full hydrogenation of the 3-hydroxy- or 3-*O*-methyl-4'-methoxy derivatives with palladium-on-charcoal furnished the desired 2- or 4-phenethyl-substituted compounds (Scheme 5). The products were formed under 20 bar H_2 in ethyl acetate in excellent yields.



Scheme 5

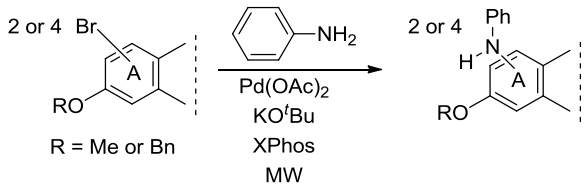
3.10. We have developed an efficient protocol for the Buchwald–Hartwig amination of 2-bromo-3-*O*-methyl-13α-estrone with aniline (Scheme 6). An optimization process was performed by the systematic change of catalyst, base and ancillary ligand. We found that the outcome of the couplings greatly depends on the applied conditions. A comparison of conventional heating and microwave-assisted heat transfer was investigated observing significantly shortened reactions under microwave irradiation. Optimal reaction conditions were identified. The highest yields were achieved by using $Pd(OAc)_2$ as catalyst, KO^{t}Bu as base and XPhos as ancillary ligand at 100 °C in a microwave reactor.

3.11. With the best reaction conditions in hand (discussed in 3.10.), the couplings at C-2 of 2-bromo-3-*O*-methyl-13α-estrone were extended to monosubstituted anilines bearing electronically different substituents at *otho*, *meta* or *para* positions (Scheme 6). All couplings proceeded with high yields. The best yields were achieved with nitroanilines, irrespective of the position of the nitro group. Reactions of methylanilines led to slightly lower yields, indicating that the presence of the electron-donating methyl group is less advantageous.



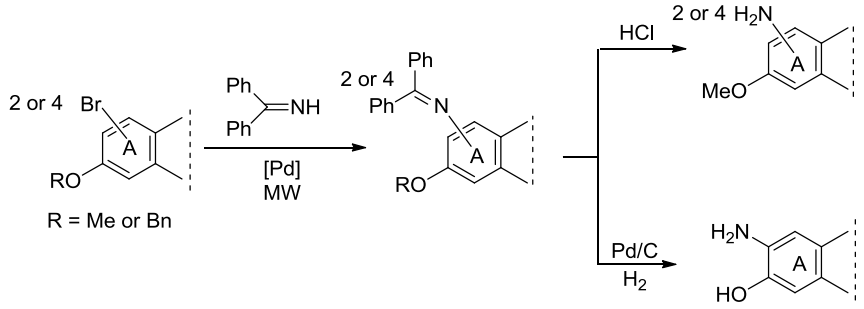
Scheme 6

3.12. The coupling at C-4 of 4-bromo-3-*O*-methyl-13α-estrone with aniline under the conditions mentioned above yielded the 4-phenylamino derivative in high yield (Scheme 7). Irrespective of the more bulky nature of the benzyl ether group, both 3-*O*-benzyl-2-bromo- and -4-bromo-13α-estrone were successfully aminated without the need for changing the reaction conditions established for couplings at C-2.



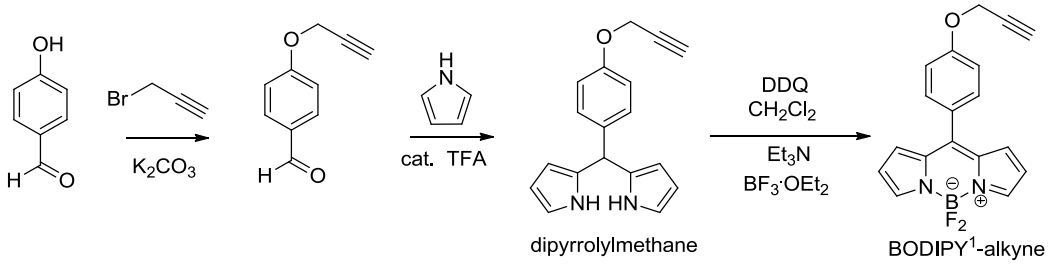
Scheme 7

3.13. The efficient C(sp^2)–N coupling method elaborated in 3.10. proved to be suitable for the reaction of 2- or 4-bromo regioisomers in both the 3-*O*-methyl and 3-*O*-benzyl series with benzophenone imine as an amine precursor (Scheme 8). The resulting 2- or 4-(*N*-diphenylmethylideneamino)-13α-estrones could efficiently be transformed into the appropriate amino counterparts. Deprotection was achieved by Brønsted acid in the case of 3-methyl ethers and via hydrogenolysis from 3-benzyl ethers using palladium-on-charcoal catalyst. Concerning 3-benzyl ethers, deprotection of the 3-OH and liberation of the 2-NH_2 functions occurred simultaneously. Thus, the combination of the elaborated convenient C(sp^2)–N coupling procedure with deprotection led to the desired 2-amino-13α-estrone in only two steps with high overall yield.



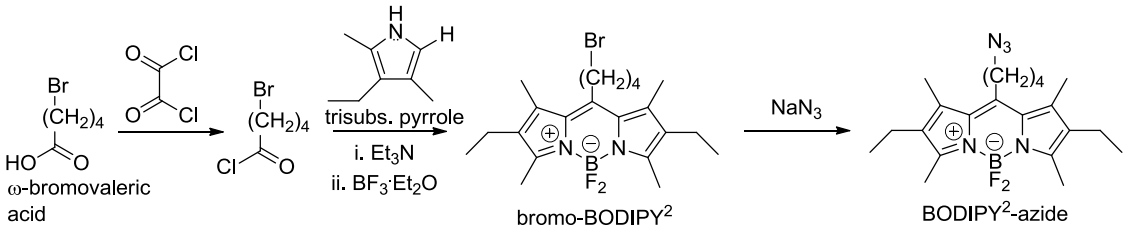
Scheme 8

3.14. BODIPY[1]-alkyne a known compound was synthesized using the aldehyde–pyrrole condensation strategy by newly established procedure based on the modification and combination of literature methods (Scheme 9). After the propargylation of *para*-hydroxybenzaldehyde, the dipyrrolylmethane core was built via trifluoroacetic acid (TFA)-catalyzed condensation of the formed aldehyde and pyrrole. Subsequent oxidation of dipyrrolylmethane with DDQ (2,3-dichloro-5,6-dicyano-*para*-benzoquinone) and complexation with $BF_3{\cdot}OEt_2$ furnished the desired BODIPY[1]-alkyne in high yield.



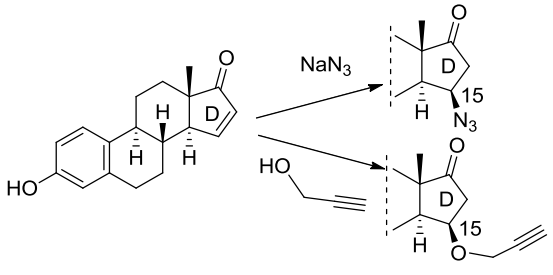
Scheme 9

3.15. A new BODIPY derivative bearing the azide function was synthesized using the acyl chloride–pyrrole condensation strategy (Scheme 10). Trisubstituted pyrrole was selected for the condensation in order to avoid the formation of side-products. ω-Bromovaleric acid was used as the azide precursor in order to build in a four-carbon-long spacer chain to the conjugate. The required acyl chloride was formed *in situ* with oxalyl chloride. Condensation of the acyl chloride and pyrrole allowed the selective formation of dipyrrolylmethene. Subsequent complexation with $BF_3{\cdot}OEt_2$ without isolation of the intermediate led to the bromo-BODIPY^2 product in high yield. The new BODIPY^2-azide was formed in a bromine-to-azide exchange reaction.



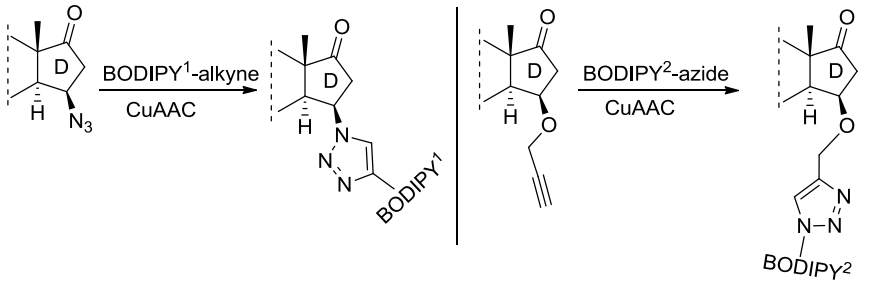
Scheme 10

3.16. The $\Delta^{15,16}$-estrone was transformed into the appropriate alkyne or azide derivative by Michael addition (Scheme 11). The terminal alkyne function was introduced onto C-15 via *O*-propargylation with propargyl alcohol in dichloromethane, using a catalytic amount of NaOH as the base. The 15-azide derivative was synthesized via the addition of HN_3 formed *in situ* from NaN_3 and acetic acid. The nucleophilic attack onto C-15 occurred in a stereoselective fashion in both cases leading to the formation of a single stereoisomer (15β). The 15β orientation of the *O*-propargyl substituent was deduced from the NOESY NMR spectrum showing a cross-peak between the signals of 15α-H and 16α-H.



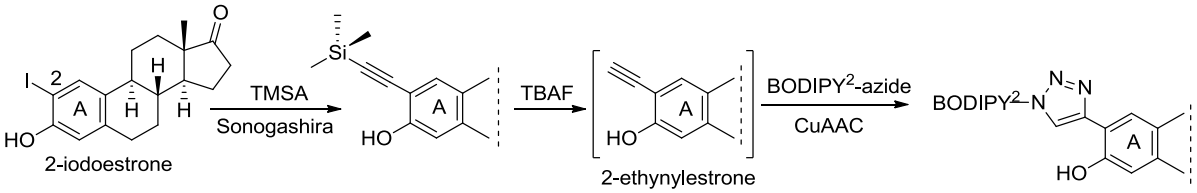
Scheme 11

3.17. With estrone and BODIPY alkyne and azide in hand, we carried out Cu(I)-catalyzed azide–alkyne conjugation (CuAAC) reactions in order to synthesize the desired fluorescent estrone conjugates (Scheme 12). CuAAC conditions were applied according to the protocol developed previously by our research group. Conjugations were achieved using CuI as the catalyst, PPh_3 as the ligand, and DIPEA as the base under conventional heating in toluene solvent. Reactions led to the desired fluorescent estrone-BODIPY conjugates in excellent yields.



Scheme 12

3.18. Fluorescent labelling of estrone through ring A was achieved (Scheme 13). The terminal alkyne function was introduced directly onto the C-2 position in two steps. Sonogashira coupling of 2-iodoestrone with trimethylsilylacetylene (TMSA) was performed via our microwave-assisted procedure established earlier (see 3.5.). Deprotection of 2-trimethylsilylethynyl-estrone was carried out with tetrabutylammonium fluoride (TBAF) *in situ* during the CuAAC reaction. The fluorescent estrone-BODIPY conjugate was isolated in high yield via the procedure discussed in 3.17.



Scheme 13

4. Publications directly related to the dissertation (MTMT ID: 10052580)

1. **Bacsa I.**, Jójárt R., Schneider G., Wölfling J., Maróti P., Herman B.E., Szécsi M., Mernyák E. Synthesis of A-ring halogenated 13α-estrone derivatives as potential 17β-HSD1 inhibitors. *Steroids* **2015**, *104*, 230−236. **IF: 2.513**

2. **Bacsa I.**, Jójárt R., Wölfling J., Schneider G., Herman B.E., Szécsi M., Mernyák E. Synthesis of novel 13α-estrone derivatives by Sonogashira coupling as potential 17β-HSD1 inhibitors; *Beilstein Journal of Organic Chemistry*, **2017**, *13*, 1303−1309. **IF: 2.337**

3. **Bacsa I.**, Konc C., Orosz A. B., Kecskeméti G., Rigó R., Özvegy-Laczka C., Mernyák E. Synthesis of novel C-2 or C-15 labeled BODIPY-estrone conjugates; *Molecules*, **2018**, doi: 10.3390/molecules23040821 **IF: 2.861**

4. **Bacsa I.**, Szemerédi D., Wölfling J., Schneider G., Fekete L., Mernyák E. The first Pd-catalysed Buchwald-Hartwig aminations at C-2 or C-4 in the estrone series; *Beilstein Journal of Organic Chemistry*, **2018**, accepted for publication **IF: 2.337**

5. **Bacsa I.**, Herman B. E., Jójárt R., Herman K. S., Wölfling J., Schneider G., Varga M., Tömböly C., Rižner T. L., Szécsi M., Mernyák E. Synthesis and structure–activity relationships of 2- and/or 4-halogenated 13β- and 13α-estrone derivatives as enzyme inhibitors of estrogen biosynthesis; *Journal of Enzyme Inhibition and Medicinal Chemistry*, **2018**, submitted

Total impact factor: 10.048

5. Other publications

1. Mernyák E., Szabó J., **Bacsa I.**, Huber J., Schneider G., Minorics R., Bózsity N., Zupkó I., Varga M., Bikadi Zs., Hazai E., Wölfling J. Synthesis and antiproliferative effect of D-homo- and D-secoestrones, *Steroids*, **2014**, *87*, 128−136. **IF: 2.639**

2. Szabó J., **Bacsa I.**, Wölfling J., Schneider G., Zupkó I., Varga M., Herman B. E., Kalmár L., Szécsi M., Mernyák E., Synthesis and *in vitro* pharmacological evaluation of *N*-[(1-benzyl-1,2,3-

triazol-4-yl)methyl]-carboxamides on D-secoestrone scaffolds, *J Enzyme Inhib Med Chem*, **2016**, *31*, 574−579. **IF: 3.428**

3. Herman B.E., Szabó J., **Bacsa I.**, Wölfling J., Schneider G., Bálint M., Hetényi C., Mernyák E., Szécsi M. Comparative investigation of the *in vitro* inhibitory potencies of 13-epimeric estrones and D-secoestrones towards 17β-hydroxysteroid dehydrogenase type 1; *Journal of Enzyme Inhibition and Medicinal Chemistry* **2016**, *31*, 61−69. **IF: 3.428**

Total impact factor: 9.495

6. Lectures and posters related to the dissertation

Lectures:

Bacsa I., MTA Szteroid és Terpenoidkémiai Munkabizottsági ülés, Szeged, Hungary, 27. November 2017. *Preparation of potential enzyme inhibitors in the estrone series.*

Bacsa I., Jójárt R., MTA Szteroid- és Terpenoidkémiai Munkabizottsági előadóülés Szeged, Hungary, 11. November 2016. *Új 13α-ösztron származékok Pd-katalizált szintézise*

Bacsa I., Hungarian Science Day in Szeged, Szeged, Hungary, 8. November 2016. *Synthesis of biologically active 13α-estrone derivatives.*

Jójárt R., Bacsa I., Wölfling J., Schneider Gy., Herman B. E., Szécsi M., Mernyák E., 6th International PSU-UNS Bioscience Conference, Novi Sad, Serbia, 19-21. September 2016. *Synthesis and 17β-HSD1 inhibition of novel 2- or 4-substituted 13α-estrone derivatives.*

Bacsa I., 1st Hungarian-Norwegian Summer School on Bioactive Substance Research, Tromsø, Norway, 15 July 2016. *Aromatic halogenation of estrone.*

Bacsa I, Jójárt R, Herman BE, Schneider Gy, Wölfling J, Szécsi M, Mernyák E, 2nd Innovation in Science, Szeged, Hungary, 26. September 2015. *Synthesis of A ring substituted estrone derivatives as potential 17β-HSD1 inhibitors.*

Posters:

Bacsa I., Jójárt R., Wölfling J., Schneider Gy., Herman B. E., Szécsi M., Mernyák E., 10th Joint Meeting on Medicinal Chemistry, Dubrovnik, Croatia, 25-28. June 2017. *Synthesis of novel 13α-estrone derivatives as potential 17β-HSD1 inhibitors.*

Bacsa I., Herman K. S., Jójárt R., Wölfling J., Schneider Gy., Herman B. E., Szécsi M., Mernyák E., Annual Meeting of Hungarian Chemical Society, Hajdúszoboszló, Hungary, 19-21. June 2017. *Synthesis of 13α-estrone derivatives and their 17β-HSD1 enzyme inhibitory effect.*

Rigó R., Patik I., Bacsa I., Mernyák E., Laczka Cs., Straub-Napok, Szeged, Hungary, 24-25. May 2017. *Estrone derivates as potent inhibitors of human Organic Anion Transporter Peptides (OATP) 2B1.*

Bacsa I., Herman K. S., Jójárt R., Wölfling J., Schneider Gy., Herman B. E., Mernyák E., Szécsi M., 6th International PSU-UNS Bioscience Conference, Novi Sad, Serbia, 19-21. September 2016. *Synthesis and 17β-HSD1 inhibition of halogenated 13α-estrone derivatives.*

Herman B. E., Szabó J., Bacsa I., Wölfling J., Schneider Gy., Mernyák E., Szécsi M., Gardi J., Valkusz Zs., A Magyar Endokrinológiai és Anyagcsere Társaság XXVI. Jubileumi Kongresszusa, Szeged, Hungary, 5-7. May 2016. *In vitro enzyme activity investigation of 17β-HSD1 via 13α-estrone derivatives.*

Bacsa I., Jójárt R, Herman BE, Schneider Gy, Wölfling J, Szécsi M, Mernyák E, MKE 2nd National Conference, Hajdúszoboszló, Hungary, 31th August – 1st September 2015. *Perparation of novel 13α-estrone conjugates with Sonogashira coupling.*

Bacsa I., Jójárt R, Herman BE, Schneider Gy, Wölfling J, Szécsi M, Mernyák E, 9th Joint Meeting in Medicinal Chemistry– Athens, Greece, 7–10. June 2015. *Synthesis of novel 13α-estrone derivatives as 17β-HSD1 inhibitors.*

7. Lectures and posters not related to the dissertation

Lecture:

Bacsa I., Szabó J., Wölfling J., Schneider Gy., Zupkó I., Mernyák E., 1st Innovation in Science – Doctoral Student Conference, Szeged, Hungary, 2–3. May 2014. *Snythesis of novel steroid-alkynes and their in vitro pharmacologycal examination.*

Bacsa I., National Scientific Conference for Students, Szeged, Hungary, 29. April 2014. *Synthesis of nitrogen containing, antitumoral D-secoestrones.*

Bacsa I., XXX. Anniversary National Scientific Conference for Students, Pécs, Hungary, 28. April 2011. *Preparation of hystidine-rich proteins for accumulation of transition metal ions with biotechnologycal techniques.*

Poster:

Szabó J., Bacsa I., Wölfling J., Schneider Gy., Zupkó I., Mernyák E., 20th International Conference on Organic Synthesis, Budapest, Hungary, 29th June – 4th July 2014. *Synthesis of novel antiproliferative D-secoalkyes of 13α- and 13β-estrone and their heterocyclic derivatives.*