In vitro inhibition of the estrogen biosynthesis enzyme system with new steroid derivatives

The theses of Ph.D. dissertation

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1 Introduction and aims

In the beginning of biosynthetic cascade of estrogens (Fig. 1), cytochrome P450 dependent 17α-hydroxylase-$C_{17,20}$-lyase enzyme ($P450_{17\alpha}$) converts C_{21} precursors, pregnenolone and progesterone first to their 17α-hydroxy derivatives 17-hydroxypregnenolone and 17-hydroxyprogesterone (17OHProg). A subsequent side chain cleavage process ($C_{17,20}$-lyase activity) forms C_{19} steroids, dehydroepiandrosterone (DHEA) and androstenedione. Androstenedione then can be converted partially to testosterone (Test).

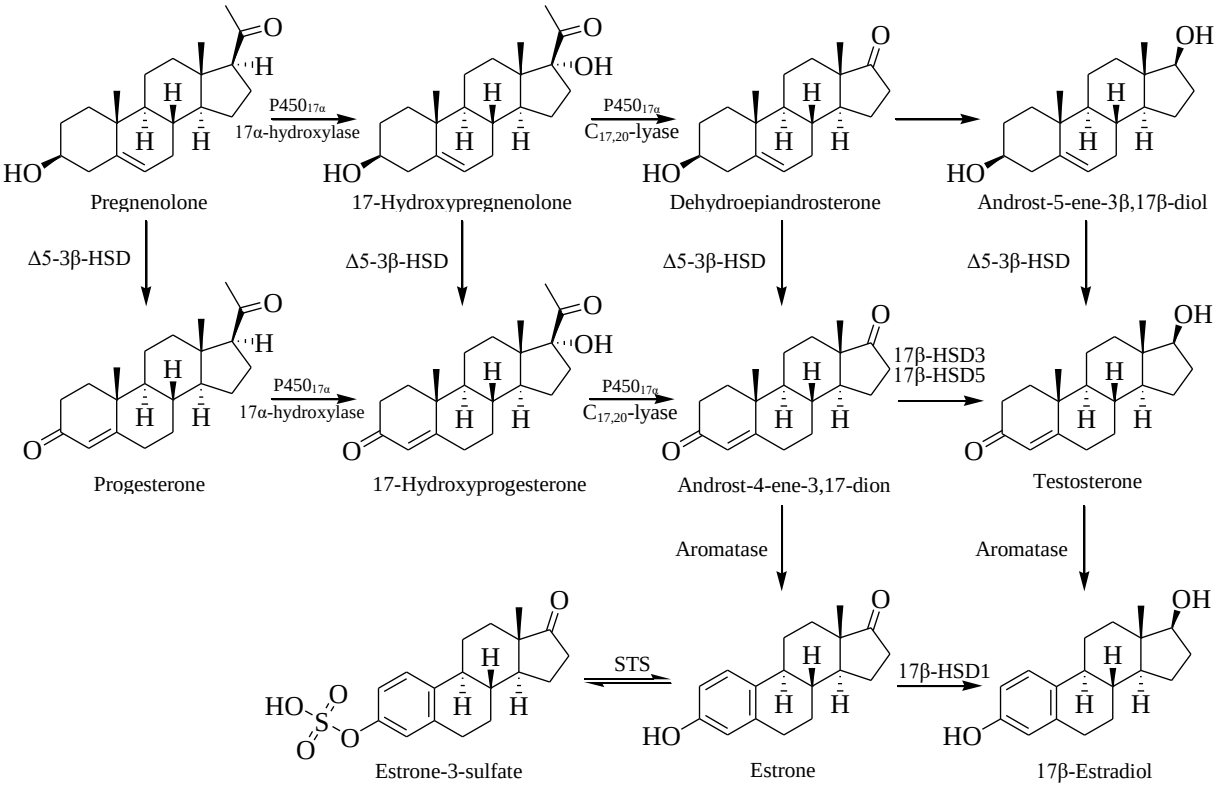


Fig. 1 Major pathways of estrogen biosynthesis

Estrone and 17β-estradiol are synthesised from androstenedione and testosterone by the cytochrome P450 dependent aromatase enzyme ($P450_{19A1}$), which performs also the cleavage of the C-19 methyl group beyond the aromatization of the steroidal A ring. Estrone is further metabolized by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) which is activating the estrogenic effect by the stereospecific reduction of the steroidal C-17 oxo function and forms the active hormone 17β-estradiol. Estrogens can be transported and stored in the form of estrone-3-sulfate. This is synthesized from estrone by the action of estrogen sulfotransferase with estrone being regenerated by the steroid sulfatase (STS)-catalysed hydrolysis of estrone-3-sulfate.

Primary biosynthesis of estrogens is under the endocrine control of the hypothalamo-hypophyseal axis and takes place mainly in functionating ovaries. Substantial production occurs

also in extragonadal sites (such as breast and adipose tissue), and this peripheral biotransformation may regulate estrogen action in an intracrine manner.

Estrogens have major role in development and progression of gynecological pathologies, among them in hormone dependent forms of breast cancer, ovarian tumors, uterine carcinomas, and neoplasms of the endometrium. Breast cancer tissues, for instance, have been shown to express enzymes of the estrogen producing machinery in exaggerated manner resulting in elevated local 17β-estradiol concentrations, which is believed to promote the pathophysiological cell proliferation. Progression of the hormone dependent tumors can be controlled by withdrawal of the estrogens. For an efficient and complete estrogen deprivation concomitant inhibition of the key enzymes in the cascade as well as blockade of the adrenal supply of prehormones *via* inhibition of the $P450_{17\alpha}$ might be necessary. Compounds possessing either specific or multiple inhibitory effect against the $P450_{17\alpha}$, the aromatase, the STS, and the 17β-HSD1 are interesting targets of the drug development.

The aim of this work was to investigate the inhibitory effect of novel steroid derivatives against key enzymes of estrogen biosynthesis: $C_{17,20}$-lyase activity of $P450_{17\alpha}$, aromatase, STS, and 17β-HSD1. Newly synthesized compounds are the results of cooperating research groups. Test compounds possessed various structural modifications applied regularly in pharmacological research for the modulation of biological effect.

We aimed to develop *in vitro* radiosubstrate incubation methods to measure aromatase, STS and 17β-HSD1 activity and inhibition. Our goal was to collect data on inhibitory potentials of new test compounds (IC_{50}) and molecular structure–activity relationships. We also planned to investigate the enzyme specificity of the inhibitors and possible multiple inhibitory effects. We also aimed to investigate kinetic and mechanistic properties of the inhibitors. Final aim of this work was to provide further information to better understanding of ligand biding mechanisms of the enzymes. Our new data might be useful for the development of novel inhibitor compounds of estrogen biosynthesis and contribute to the exploration of new drug candidates.

2 Experimental

2.1 Incubation procedures

Enzymatic incubations were carried out in the HEPES buffer medium at a final volume of 200 µl. The substrate was added to the incubate in 20 µl of a 25 v/v% propylene glycol (or acetonitrile for aromatase) in HEPES buffer solution, whereas test compounds were applied in 10 µl of dimethyl sulfoxide solution. In incubates of the inhibition studies substrate concentration was 1.0 µM. Incubations were performed at 37 °C with continuous shaking (200 rpm) and terminated by cooling and the addition of organic solvents of the subsequent extraction procedure. Radioactivity of the isolated product and remaining substrate was measured by means of liquid scintillation counting (Packard Tri-Carb 2200CA). Enzyme activity was calculated from the amount of the product formed with correction of the background and recovery. Control samples with no inhibitor and blank samples were incubated simultaneously. Technical details of enzyme activity measurements are listed in Table 1.

Table 1. Description of radiosubstrate enzyme incubation methods used for inhibition tests (*from Szabó et al. *Acta Biol Hung*, **2015**, *66*, 274–281)

Enzyme	**$C_{17,20}$-lyase***	**Aromatase**	**STS**	**17β-HSD1**
Enzyme source	Rat testicular homogenate	Human placental microsomes	Human placental microsomes	Human placental cytosol
Substrate	17OHProg	Testosterone	Estrone-3-sulfate	Estrone
Product	Androst-4-ene-3,17-dione	17β-Estradiol	Estrone	17β-Estradiol
Coenzyme	NADPH	NADPH	-	NADPH/NADH
Incubation time	20 min	40 min	20 min	2.5 min
Enzyme amount in mg tissue equivalent	3.2 mg	20 mg	0.2 mg	0.4 mg
Radioactivity of the tracer (dpm/incubate)	170000	250000	30000	250000
Product isolation technique	TLC	Extraction	Extraction	TLC

2.2 Inhibition studies

Relative conversions (RC-s) compared to non-inhibited controls (100%) were determined. The assays were performed in triplicates for the determination of the percentages of inhibited RC-s at a final inhibitor concentration of 10 µM or 50 µM. Mean of the inhibited RC-s and the standard deviations (SD-s) were calculated. IC_{50} values (the inhibitor concentration, which decreases enzyme activity to 50%) were determined for the most effective and other selected

test compounds. IC_{50} of unlabeled substrates and reference compounds were measured as reference. Relative inhibitory potentials (RIP) of the test compounds were calculated using reference IC_{50} data measured with the corresponding cofactor: RIP= IC_{50} of test compound / IC_{50} of unlabeled substrate.

2.2.1 Kinetic analyses

The Michaelis constant (K_M) of substrates was measured using increasing concentration of the unlabeled substrate. The K_M was calculated from Michaelis-Menten analysis (reaction velocity vs. substrate concentration) using the GraphPad Prism 4.0 software. Inhibition constant (K_i) was determined *via* measurement of the enzymatic transformation using different fixed substrate concentrations and varied inhibitor concentrations. The Dixon's linear transformation (1/velocity *vs*. inhibitor concentration) was applied for evaluation and K_i was estimated from abscissa of intersection of the lines of different substrate concentrations. Mechanism of binding was identified according to the Dixon's graphs and the secondary plot of slopes of the Dixon's lines *vs.* 1/substrate concentration.

2.2.2 Reversibility studies

2.2.2.1 Charcoal adsorption method

Reversibility of potent $C_{17,20}$-lyase inhibitors was investigated *via* preincubation and charcoal adsorption method. Testicular homogenate in incubation mixture (200 µl) was preincubated with high concentration (10 µM and 50 µM) of the tested compounds, in the presence of NADPH cofactor for 10 and 40 minutes at 37 ºC. After the addition of 50 µl 3% dextran coated charcoal suspension in 0.6% final concentration, the preincubated samples were kept on ice for 15 minutes and centrifuged at 4 ºC. Supernatants were drained and used for inhibition tests.

2.2.2.2 Dilution method

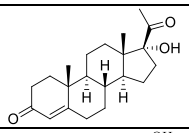
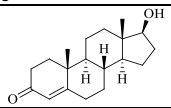
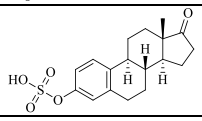
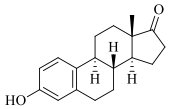
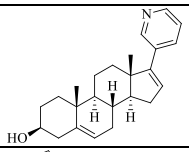
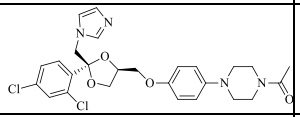
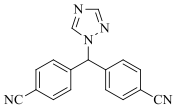
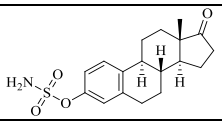
Mechanistic studies for STS and 17β-HSD1 inhibitors were performed *via* preincubation and dilution method. The inhibitor compounds were preincubated with the microsoma fraction or placental cytosol in a volume of 4.0 µl at 37 °C for various time periods (2.5-20 min). Following this procedure samples were diluted with the incubation medium to 50-fold. Enzyme activity measurements were started immediately for the 17β-HSD1 and for STS after a 20 min secondary incubation time to allow dissociation. The enzymatic reactions were started by the addition of the substrate, and subsequent incubation procedures were then carried out as described. Conversions were compared to their respective controls, which were treated in a similar way but were made without inhibitors.

3 Results

3.1 Methodological results

In the methodological developments we established *in vitro* radiosubstrate incubation methods to measure aromatase, STS and 17β-HSD1 activity and inhibition [II.; III.; VII.; IX.]. We adapted and improved extraction techniques for enzyme products of aromatase and STS and developed a TLC separation for the substrate and product of 17β-HSD1. These isolation techniques provided high recovery, low blank and good reproducibility. Incubation protocols were suitable for rapid testing of numerous compounds. Parameters characterizing substrate affinity values (K_M and IC_{50}) were measured and potential of reference inhibitors were determined. Reference parameters for the investigated enzymes are shown in Table 2.

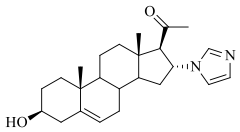
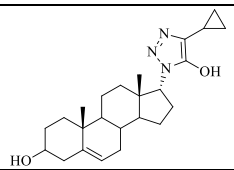
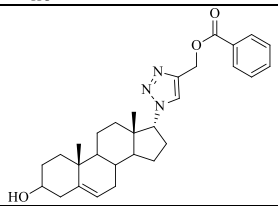
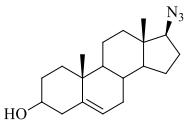
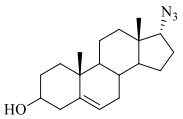
Table 2. Parameters of reference compounds for the investigated enzymes (*from Szabó et al. *Acta Biol Hung*, **2015**, *66*, 274–281)

Comp.	Structure	$C_{17,20}$-lyase*	Aromatase	STS	17β-HSD1
		IC_{50} and/or $K_{M/i}$±SD (μM) or RC at 10 μM±SD (%)			
17OHProg		IC_{50}= 1.6±0.35			
Test			IC_{50}= 0.52±0.14 K_M= 0.60±0.18		
Estrone-3-sulfate			87±4	IC_{50}= 5.2±1.2 K_M= 8.5±2.9	80±5
Estrone			78±7	63±2 IC_{50}= 24.1±10.2	IC_{50}= 0.63±0.11 (NADPH) K_M= 0.17±0.040 (NADPH) IC_{50}= 2.0±0.18 (NADH)
Abiraterone		IC_{50}= 0.0125±0.0015			
Ketoconazole		IC_{50}= 0.32±0.02	IC_{50}= 14.2±0.40		
Letrozole			IC_{50}= 0.0038±0.0010		
EMATE			95±8	IC_{50}= 0.0098±0.0038 K_i= 0.0044±0.0005	IC_{50}= 4.6±2.9

3.2 Investigations on inhibition of $C_{17,20}$-lyase activity of $P450_{17\alpha}$ with steroid derivatives substituted on ring D

We investigated $C_{17,20}$-lyase inhibitory effect of novel 16α-amino-pregnenolone and 17-triazolyl-androst-5-en-3β-ol compounds [IV.; VI.]. Several potent inhibitors were identified and results revealed that stereoisomerism of the substituent at the C-17 influenced the $C_{17,20}$-lyase inhibitory effect markedly. Results also verified that extension of side chains on position C-16 and C-17 may improve inhibitory potential. We found the parent compound 17β-azido-androst-5-en-3β-ol to be a potent and irreversible $C_{17,20}$-lyase inhibitor, whereas aromatase (another cytochrome P450 dependent enzyme in the estrogen biosynthesis) was not inhibited by this compound. These new findings indicate that azido group on the steroidal C-17 is a suitable pharmacophore in the inhibition of the $P450_{17\alpha}$ worth for further investigations. Inhibitory potentials of most potent 16α-amino-pregnenolone, 17-triazolyl- and 17β-azido-androst-5-en-3β-ol inhibitors of $C_{17,20}$-lyase are shown in Table 3.

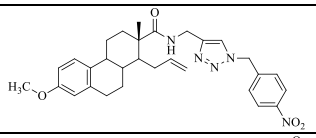
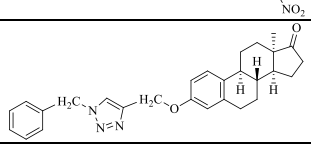
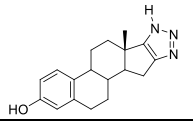
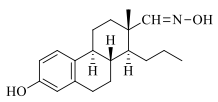
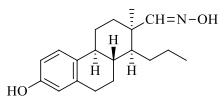
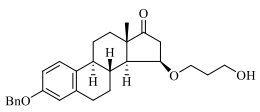
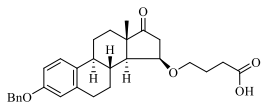
Table 3. Inhibitory potentials of most potent 16α-amino-pregnenolone, 17-triazolyl- and 17-azido-androst-5-en-3β-ol inhibitors of $C_{17,20}$-lyase

Comp.	Structure	IC_{50}±SD (μM)	RC at 10 μM±SD (%)	RC at 50 μM±SD (%)
ML-AD-46		1.8±0.36	21±1	19±2
GT-166		3.1±1.4	28±3	15±2
GT-70		3.5±0.8	32±5	6±1
GT-101		0.60±0.18	15±2	17±3
SCH-1737		21.2±6.8	64±9	42±4

3.3 Comparative investigations on cofactor dependent inhibition of 17β-HSD1

We investigated 17β-HSD1 inhibitory potential of several groups of diverse new steroidal compounds giving particular attention to the cofactor dependence of the presumed inhibitory effect [II.; III.; VIII.]. We identified numerous potent inhibitors and results revealed that the apparent *in vitro* potentials obtained with the cofactors NADPH or NADH may differ substantially. This finding indicates that binding of the phosphorylated or the unphosphorylated cofactors may exert different influence on structural elements of the substrate binding site which are involved in inhibitor binding. Results measured in the presence of NADH are less relevant to the potential *in vivo* effect, therefore these data must be evaluated with caution in inhibitor optimization and in lead selection. Comparative investigations on cofactor dependent inhibition offer a good experimental tool for mechanistic studies and in combination with theorethical methods may provide a better insight to the mechanisms of inhibitor binding of the 17β-HSD1. Examples of the compounds investigated towards cofactor dependent 17β-HSD1 inhibitory effect are shown in Table 4.

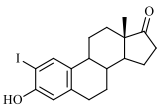
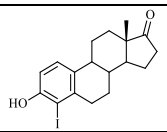
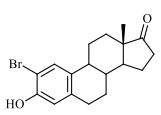
Table 4. Examples of the compounds investigated towards cofactor dependent 17β-HSD1 inhibitory effect

Comp.	Structure	NADPH			NADH		
		IC_{50} ± SD (μM)	RC at 10μM ± SD (%)	RIP	IC_{50} ± SD (μM)	RC at 10μM ± SD (%)	RIP
BI-28		> 10	99±1	> 15	1.3±0.4	25±2	0.65
PZ-8		7.8±1.4		13.4	9.7±2.8		4.85
MS/AN-3		> 10	79±16	> 15	2.6±1.0		1.3
FG-7		0.070±0.027		0.11	0.077±0.036		0.039
FG-4		30±7		48	0.058±0.044		0.029
SCH-1478		0.78 ± 0.30		1.2	> 10	76 ± 7	> 5
SZE-5012		> 10	87 ± 4	> 15	3.5 ± 1.0		1.8

3.4 Inhibition of aromatase, STS and 17β-HSD1 with halogenated estrone analogs

We investigated aromatase, STS and 17β-HSD1 inhibitory effect of A-ring halogenated estrone, 13α-estrone and 17-deoxy-13α-estrone derivatives [I.; VII.]. We identified numerous potent inhibitors exerting specific or dual inhibitory effect. 2-Iodoestrone was found to be a specific and highly potent 17β-HSD1 inhibitor, whereas its 4-iodo counterpart has dual inhibition against STS and 17β-HSD1. Our results reveal that 2- and 4-bromoestrone can be also regarded as dual inhibitors against STS and 17β-HSD1. The inverted 13α-configuration and absence of the 17-oxo function decreases the inhibitory potentials in various extents against aromatase, STS and 17β-HSD1 of most of the A-ring halogenated compounds, however, their effect still remained substantial. Our results obtained for aromatase, STS and 17β-HSD1 inhibitory effect of halogenated estrone analogs revealed that substituens in the position C-2 and/or C-4 and polarization of the phenolic OH in the position C-3 of ring A, as well as structural features of the ring D determine the binding affinity to the enzymes cooperatively. This complex binding mechanism seems to be relevant for the investigated enzymes owning rather different structures and operating distinct catalytic mechanisms. This complex binding mechanism is also applicable for the inhibitor compounds possessing either substrate- or product-like structures. Submicromolar IC_{50} parameters of these bromo and iodo substituted estrones together with their reduced estrogenicity known from literature indicate that these derivatives can be suitable bases for design of lead compounds in drug development. Our results reveal that cumulative xenobiotic effect of halogenated estrone derivatives exerted on the estrogen biosynthesis and the potential endocrine disrupting effect can be a matter of concern. Most potent inhibitor compounds are shown in Table 5.

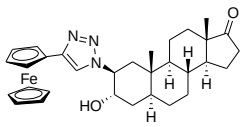
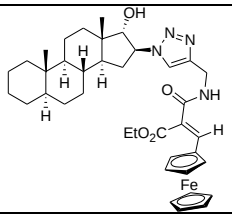
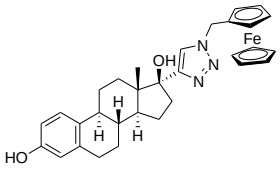
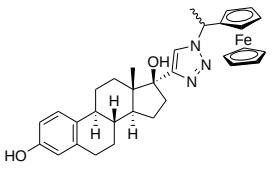
Table 5. Most potent A-ring halogenated estrone derivatives

Comp.	Structure	Inhibition of enzyme activities RC±SD (%), IC_{50}±SD (μM) and K_i±SD (μM)		
		Aromatase	STS	17β-HSD1
EM-1048V		62±1	64±3	IC_{50}= 0.064±0.034
EM-1048III		88±2	IC_{50}= 0.23±0.09 K_i= 0.36±0.05	IC_{50}= 0.36±0.25
EM-1049I		IC_{50}= 8.7±2.8	IC_{50}= 2.0±0.4	IC_{50}= 0.095±0.031

3.5 Inhibition of aromatase, 17β-HSD1 and STS with steroidal ferrocenes

We investigated inhibitory effects of diverse steroidal ferrocene substances exerted on aromatase, STS and 17β-HSD1 [IX.]. Test compounds were found weakly effective against aromatase and 17β-HSD1, nevertheless, certain triazolyl-ferrocene derivatives exerted potent inhibitions against STS and displayed higher affinities to this enzyme than the substrate estrone-3-sulfate did. Kinetic and mechanistic investigations revealed that the compound bearing triazolyl-ferrocene substituent in the C-2 position was bound in a reversible manner, whereas the C-16 and C-17 derivatives were irreversible inhibitors. Experiments demonstrated a key role of the ferrocenyl moiety in the enhanced binding affinity of the inhibitors. Our most potent STS inhibitors, the 17α-triazolyl-ferrocene derivatives of 17β-estradiol belong to the group of the most effective STS inhibitors published so far. A presumed synergism between the suppressed estrogen dependent cell proliferation evolved by the STS inhibition and hormone-independent cytotoxicity known from the literature can make this type of compounds particularly promising drug candidates for the pharmacological therapy of hormone dependent gynecological cancers. Most potent steroidal ferrocene derivatives are shown in Table 6.

Table 6. Most potent steroidal ferrocenes and their inhibitory potentials

Comp.	Structure	Inhibition of enzyme activities RC±SD (%), IC_{50}±SD (μM) and K_i±SD (μM)		
		Aromatase	STS	17β-HSD1
KL-105		100±11	41±5 IC_{50}= 4.6±1.5	81±8
KL-114		89±2	42±3 IC_{50}= 2.4±1.1	91±10
E-IL-249/5		89±8	8±2 IC_{50}= 0.084±0.043 K_i= 0.066±0.009	98±10
EV-95/3		117±15	1.0±0.5 IC_{50}= 0.035±0.006 K_i= 0.021±0.005	76±6

4 Summary

The aim of this work was to investigate the inhibitory effect of novel steroid derivatives exerted on $C_{17,20}$-lyase, aromatase, STS and 17β-HSD1, the key enzymes of estrogen biosynthesis.

1. In the methodological developments we established *in vitro* radiosubstrate incubation methods to measure aromatase, STS and 17β-HSD1 activity and inhibition. We adapted and improved isolation techniques for enzyme products. New methodologies made possible rapid testing of numerous compounds.

2. We identified potent inhibitors of $C_{17,20}$-lyase activity of the $P450_{17\alpha}$ in the series of 16α-amino-pregnenolone and 17-triazolyl-androst-5-en-3β-ol compounds. Results revealed that extension of side chains on position C-16 and C-17 could improve inhibitory potential, and stereoisomerism of the substituent at the C-17 influenced the $C_{17,20}$-lyase inhibitory effect markedly.

3. We found that 17β-azido-androst-5-en-3β-ol was a potent irreversible $C_{17,20}$-lyase inhibitor, whereas aromatase (another cytochrome P450 dependent enzyme in the estrogen biosynthesis) was not inhibited by this compound. These findings indicate that azido group on the steroidal C-17 is a suitable pharmacophore in the development of $P450_{17\alpha}$ inhibitors.

4. We identified numerous potent 17β-HSD1 inhibitors in several diverse groups of compounds (triazole and tetrazole estrone derivatives, D-secoestrone compounds and C-15 substituted estrones) and results revealed that the apparent *in vitro* potentials obtained with the cofactors NADPH or NADH may differ substantially. This finding indicates that binding of the phosphorylated or the unphosphorylated cofactors may exert different influence on structural elements of the substrate binding site which are involved in inhibitor binding.

5. We found that comparative investigations on cofactor dependent inhibition offer a good experimental tool for mechanistic studies and in combination with theorethical methods may provide a better insight to the mechanisms of inhibitor binding of the 17β-HSD1.

6. We identified numerous potent inhibitors in the series of A-ring halogenated estrone, 13α-estrone and 17-deoxy-13α-estrone derivatives exerting specific or dual inhibitory effect against aromatase, STS and 17β-HSD1. 2-Iodoestrone was found to be a specific and highly potent 17β-HSD1 inhibitor, the 2- and 4-bromoestrone and the 4-iodoestrone have dual inhibition against STS and 17β-HSD1. The inverted 13α-configuration and absence of the 17-oxo function decreases the inhibitory potentials in various extents against aromatase, STS and 17β-HSD1 of most of the A-ring halogenated compounds, however, their effect still remained substantial.

7. Our results obtained for aromatase, STS and 17β-HSD1 inhibitory effect of halogenated estrone analogs revealed that substituens in the position C-2 and/or C-4 and polarization of the phenolic OH in the position C-3 of ring A, as well as structural features of the ring D determine the binding affinity to the enzymes cooperatively. This complex binding mechanism seems to be relevant for the investigated enzymes owning rather different structures and operating distinct catalytic mechanisms. This complex binding mechanism is also applicable for the inhibitor compounds possessing either substrate- or product-like structures.

8. We found that the 2-iodoestrone is one of the most effective 17β-HSD1 inhibitors, whereas the 4-iodoestrone belongs to the group of the most potent steroidal STS inhibitors published so far. These most potent compounds and submicromolar IC_{50} parameters of other halogenated estrone analogs together with their reduced estrogenicity known from literature indicate that these derivatives can be suitable bases for design of lead compounds in drug development. Our results reveal that cumulative xenobiotic effect of halogenated estrone derivatives exerted on the estrogen biosynthesis and the potential endocrine disrupting effect can be a matter of concern.

9. We identified steroidal ferrocene compounds to be potent inhibitors of STS and the key role of the ferrocenyl moiety in the enhanced binding affinity was also demonstrated. The most effective inhibitors, 17α-triazolyl-ferrocene derivatives of 17β-estradiol belong to the group of the most effective STS inhibitors published so far. STS inhibitory effect was found to be specific regarding the other two key enzymes of the estrogen biosynthesis, aromatase and 17β-HSD1.

10. Kinetic and mechanistic investigations revealed that the compound bearing triazolyl-ferrocene substituent in the C-2 position was bound in a reversible manner, whereas the C-16 and C-17 derivatives were irreversible inhibitors.

Investigations of this work identified numerous compounds exerting potent inhibitory effect against the key enzymes involved in the estrogen biosynthesis. Relations between molecular structure–inhibitory potential were observed and binding mechanism of inhibitors were proposed. Our new achievements may contribute to the development of new drug candidates to be applied for the suppression of estrogen biosynthesis.

5 Publications related to the thesis (IF= 26.142)

I. Bacsa I, Jójárt R, Schneider G, Wölfling J, Maróti P, **Herman BE**, 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**

II. Szabó J, Bacsa I, Wölfling J, Schneider Gy, Zupkó I, Varga M, **Herman BE**, 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 Enzym Inhib Med C*, **2016**, *31*, 574–579. **IF: 4.293**

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VIII. **Herman BE**, Kiss A, Wölfling J, Mernyák E, Szécsi M, Schneider Gy: Synthesis of substituted 15β-alkoxy estrone derivatives and their cofactor-dependent inhibitory effect on 17β-HSD1, *J Enzym Inhib Med C*, **2019**, DOI: 10.1080/14756366.2019.1634064 (accepted) **IF: 4.027 (2018)**

IX. **Herman BE**, Gardi J, Julesz J, Tömböly Cs, Szánti-Pintér E, Fehér K, Skoda-Földes R, Szécsi M: Steroidal ferrocenes as potential enzyme inhibitors of the estrogen biosynthesis, *Biol Fut*, **2019** (submitted)

6 Publications not related to the thesis (IF= 4.443)

1. Klisurić OR, Szécsi M, Djurendić EA, Szabó N, **Herman BE**, Jovanović-Šanta SS, Dojčinović-Vujašković SV, Nikolić AR, Pavlović KJ, Ajduković JJ, Oklješa AM, Petri ET, Kojić VV, Sakač MN, Penov Gaši KM: Structural analysis and biomedical potential of novel salicyloyloxy estrane derivatives synthesized by microwave irradiation, *Struct Chem*, **2016**, *27*, 947–960. **IF: 1.582**

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10. Bacsa I, Jójárt R, **Herman BE**, Schneider Gy, Wölfling J, Szécsi M, Mernyák E: A-gyűrűben szubsztituált ösztron származékok, mint potenciális 17β-HSD1 inhibitorok szintézise, *Innováció a Természettudományban – Doktorandusz Konferencia*, Szeged, Hungary, **2015**.

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