Summary of Ph.D Thesis

REGIOSELECTIVE SYNTHESIS OF AZA- AND OXACYCLIC COMPOUNDS IN THE ESTRANE SERIES

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1. Introduction and aims

Steroids are a family of compounds bearing a polycondensed cyclopentano-perhydrophenanthrene skeleton, and they play a key role in maintaining homeostasis in the healthy human body. The quality and quantity, position and sterical properties of the functional groups on the sterane core can significantly alter their biological behaviour. One of the important goals of modern medicinal chemistry is the development of new, (semi)synthetic steroid derivatives, which can be used to successfully treat various types of cancer. By strategically modifying certain parts of the sterane skeleton, it is possible to minimize or entirely eliminate the unwanted hormonal side effects, thus create new, steroid-based drugs with potential antiproliferative or direct cytotoxic activity.

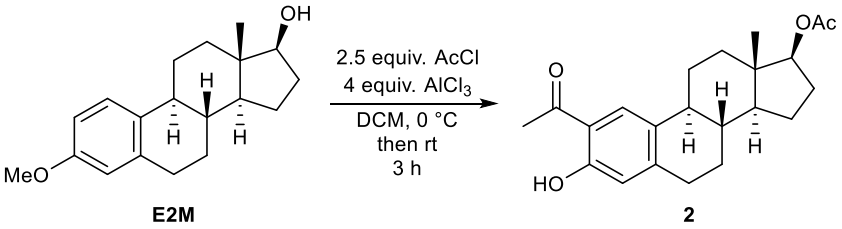
Estrone or estradiol may be an excellent choice for these purposes, as numerous examples of anticancer compounds bearing the estrane core can be found in the international literature, and our own steroidal research group have reported such derivatives in the past as well. Modification of the aromatic ring A, however, is a less researched area than the modification of ring D or steroids with a different core alltogether. For this reason, we have considered estrogens as good starting materials for further research. We were aiming to modify ring A in a regioselective manner, to explore the possibilities of multicomponent reactions, and to produce flavonoid-steroid hybrids. We also wanted to isolate the pure compounds and to optimize the reaction conditions, as well as to verify the structure of the aforementioned molecules via spectroscopic means. All compounds were planned to undergo *in vitro* pharmacological studies, performed by our partners, and we were hoping to describe any structural-activity relationships and/or substituent effects, should they appear.

2. Experimental methods

Almost all of the reactions have been performed on a millimolar scale, except for the key starting materials required for later syntheses which have been prepared on a multigram scale. The reactions have been monitored *via* thin-layer chromatography (TLC), and in select cases, heating was performed by irradiating the reaction mixture in a microwave (MW) reactor. The prepared compounds were purified by column chromatography, and the structures of the molecules were confirmed by ^{1}H-, ^{13}C-NMR and ESI-MS measurements.

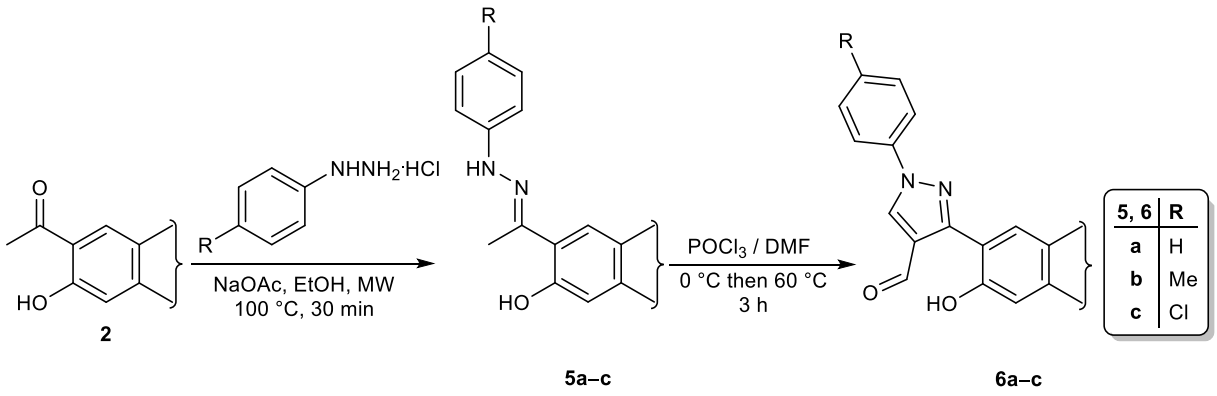
3. Novel scientific results*

3.1. 2-Acetyl-estradiol-17β-acetate (**2**), the key starting material of further transformations was prepared via the *Friedel-Crafts* acetylation and simultaneous demethylation of estradiol-3-methyl ether (**E2M**) in the presence of large amounts of $AlCl_3$. The methyl ketone was obtained in satisfactory yields (80%, *Scheme 1*).



Scheme 1

3.2. The steroidal starting material (**2**) was then reacted with various *para*-substituted phenylhydrazine hydrochloride salts in ethanol, in the presence of sodium acetate base. The reaction was performed in a MW reactor to significantly increase the conversion rate and to shorten the required reaction time compared to traditional heating techniques. The hydrazones (**5a**–**c**, 75–78%) were then subjected to *Vilsmeier-Haack* conditions to furnish the corresponding formylpyrazoles (**6a**–**c**, 72–78%, *Scheme 2*).

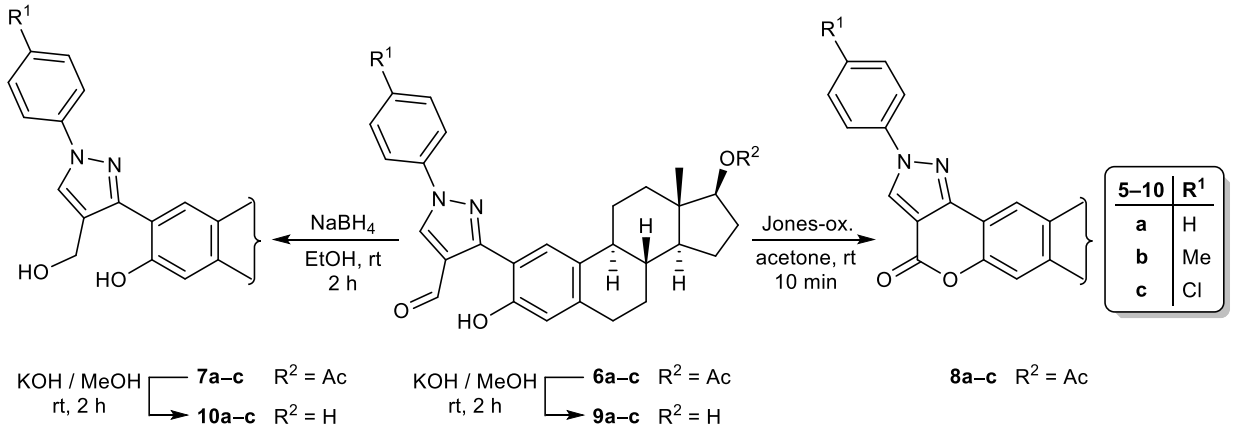


Scheme 2

3.3. To expand the number of compounds investigated, formylpyrazoles (**6a**–**c**) were subjected to redox reactions. Reduction with sodium borohydride gave primary alcohols (**7a**–**c**, 77–87%), while oxidation with *Jones*-reagent yielded pyrazolocoumarin hybrids (**8a**–**c**, 69–73%). Initially we wanted to deacetylate every compound, however, in the case of lactones the ring-opening side reaction became prevalent, and all of our attempts for recyclization have failed.

* The numbering of the compounds follows the dissertation.

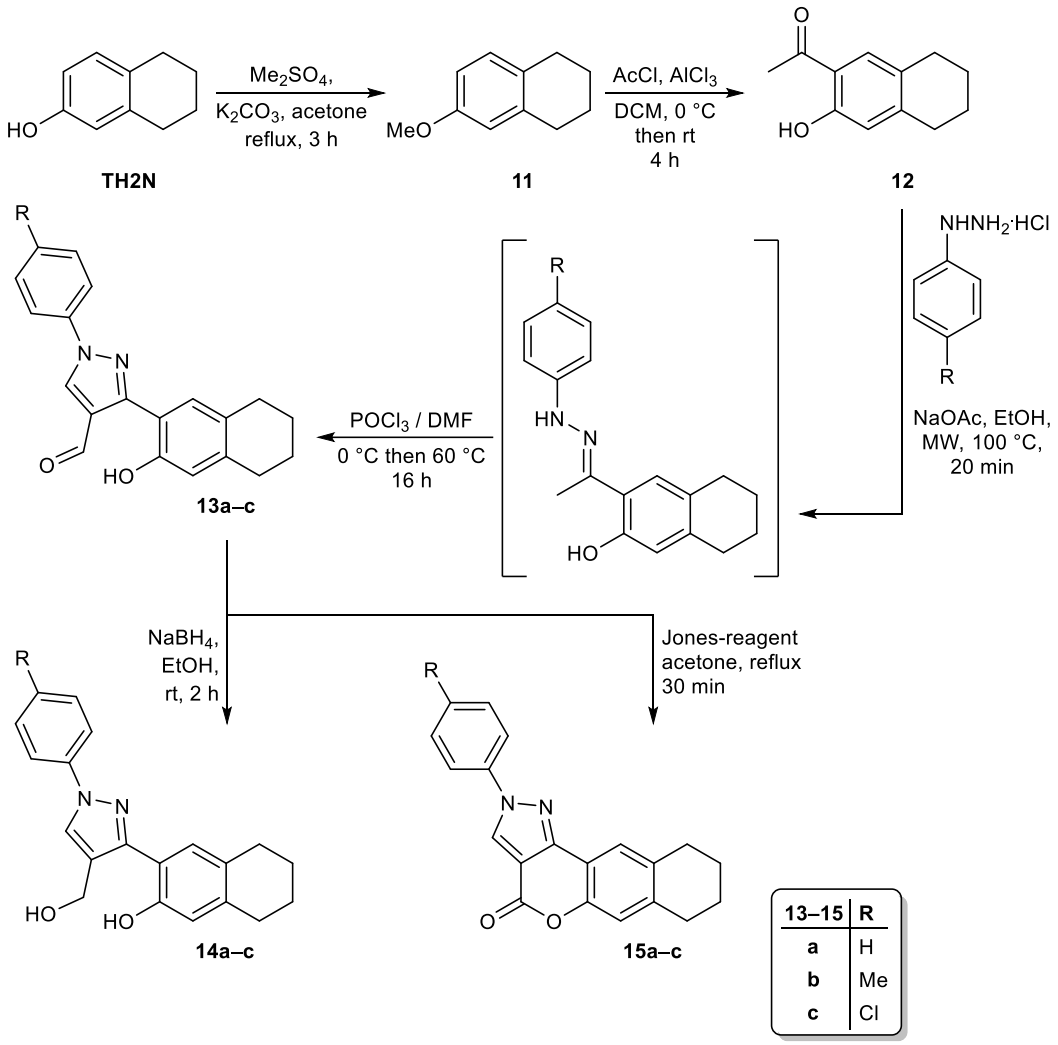
Thus, only compounds **6a**–**c** and **7a**–**c** were subjected to this transformation to furnish the desired 17-OH derivatives (**9a**–**c** and **10a**–**c**, *Scheme 3*).



Scheme 3

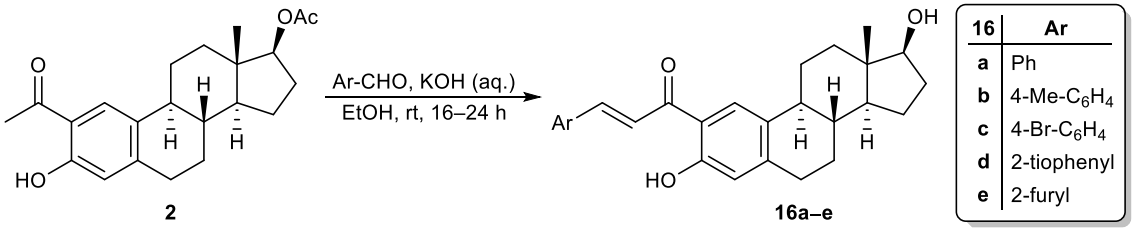
3.4. To better understand the structure-activity relationships, the aforementioned reaction scheme was expanded to include 5,6,7,8-tetrahydro-2-naphthol (**TH2N**), which served as the model of rings A and B of the estrane skeleton. Following the methylation of **TH2N** with dimethyl sulfate, compound **11** was exposed to *Friedel-Crafts* conditions to obtain the required starting material **12** in moderate yields (69%). The methyl ketone was then subjected to the same reaction conditions previously used to produce the desired hydrazones, however, the non-steroidal varieties could not be isolated, and for this reason the crude reaction mixture had to be directly subjected to *Vilsmeier-Haack* conditions. Nevertheless, the corresponding formylpyrazoles (**13a**–**c**) were obtained, but only in diminished yields (34–41%) compared to the steroidal compounds. The redox reactions have been performed in these cases as well to obtain primary alcohols **14a**–**c** (66–83%) and lactones **15a**–**c** (51–64%), but during *Jones*-oxidation, refluxing the reaction mixture was necessary for full conversion. Because of the harsher reaction conditions employed the final yields were somewhat lower as well (*Scheme 4*).

3.5. Based on the *in vitro* pharmacological results, the substitution of the aromatic benzene ring proved to be undesirable, as it decreased the selectivity in every case. Among the non-steroidal derivatives only a few compounds were effective, but their steroidal pairs were always either more selective or had a stronger antiproliferative effect. Because of this, the synthesis of further small molecular analogues was not pursued.



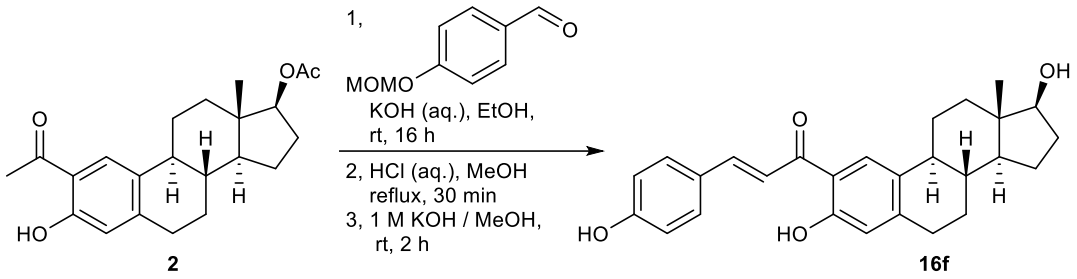
Scheme 4

3.6. 2-Acetyl-estradiol-17β-acetate (**2**) was reacted with various *para*-substituted benzaldehydes and heteroaromatic aldehydes under *Claisen-Schmidt* conditions, and the corresponding chalcones were obtained in moderate to good yields (69–86%) except for the *para*-bromo derivative **16c** (24%). Based on the NMR measurements, the main product was undoubtedly the *trans*-isomer, and owing to the strongly alkaline medium applied, the 17-acetate group underwent deacetylation in every case (*Scheme 5*).



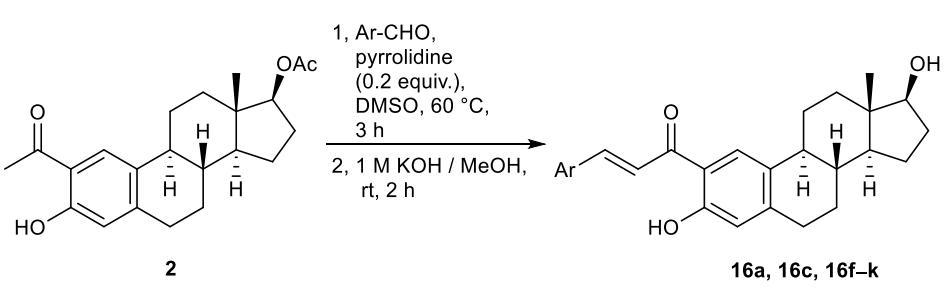
Scheme 5

3.7. Aldehydes containing phenolic OH group(s) did not react at all, even when the reaction conditions (base and solvent used, reaction temperature, etc.) were changed. However, when the methoxymethyl (MOM) protected aldehyde obtained from *para*-hydroxybenzaldehyde was applied, the desired chalcone was easily obtained (**16f**, 68%, *Scheme 6*). Major flavanone formation was observed during deprotection under acidic conditions, however, this by-product can be eliminated by a reversible ring-opening reaction under strongly basic conditions.



Scheme 6

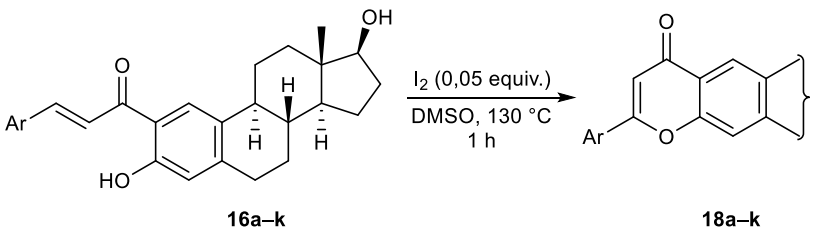
3.8. Due to the number of steps required and the use of the strongly carcinogenic chloromethyl methyl ether, we searched for an alternative synthetic route. By reacting 2-acetyl-estradiol-17β-acetate (**2**) with aromatic aldehydes containing phenolic OH group(s) in dimethyl sulfoxide, in the presence of pyrrolidine catalyst, the desired chalcones could easily be obtained. Pyrrolidine proved to be an effective catalyst for the intramolecular *oxa-Michael* addition as well, thus the reaction mixture always contained large amounts of flavanone by-products. Decomposition of the flavanone moiety with methanolic KOH was necessary in every case, but the overall yields were not affected by this phenomenon. The reactions with benzaldehyde and *para*-bromobenzaldehyde were repeated using the new reaction sequence, and in the case of **16a** a similar yield (73%) was obtained, while for **16c** the yield has more than doubled (58%). Using the pyrrolidine-DMSO system, the desired additional chalcones (**16f**–**k**) were obtained in moderate to good yields (55–79%, *Scheme 7*).



16	Ar
a	Ph
c	4-Br-C_6H_4
f	4-OH-C_6H_4
g	4-OMe-C_6H_4
h	3-OH-4-OMe-C_6H_3
i	3-OMe-4-OH-C_6H_3
j	3,4-OH-C_6H_3
k	3,4-OMe-C_6H_3

Scheme 7

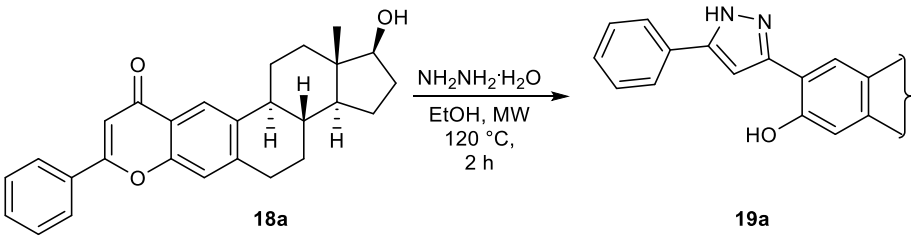
3.9. The chalcones (**16a**–**k)** underwent oxidative cyclization in DMSO in the presence of a catalytic amount of elemental iodine. During the optimization of the reaction conditions, 130 °C and 1 h were found to be the best performing combination. The corresponding flavones (**18a**–**k**) were obtained in moderate to excellent yields (66–91%, *Scheme 8*).



16, 18	Ar
a	Ph
b	4-Me-C_6H_4
c	4-Br-C_6H_4
d	2-tiophenyl
e	2-furyl
f	4-OH-C_6H_4
g	4-OMe-C_6H_4
h	3-OH-4-OMe-C_6H_3
i	3-OMe-4-OH-C_6H_3
j	3,4-OH-C_6H_3
k	3,4-OMe-C_6H_3

Scheme 8

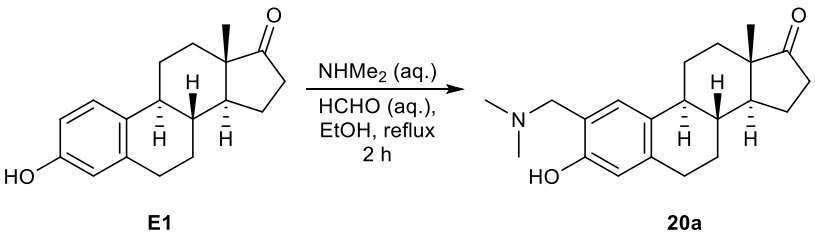
3.10. We attempted to directly transform the flavones into pyrazoles, thus compound **18a** was reacted with hydrazine hydrate. Using conventional heating techniques no conversion was observed, and while we could prepare the desired pyrazole **19a** (78%, *Scheme 9*) using MW irradiation, the transformation required prolonged exposure and a large excess of the reagent. Furthermore, **19a** demonstrated anomalous NMR behaviour, and was found to be completely ineffective during the pharmacological studies, therefore this reaction sequence was abandoned.



Scheme 9

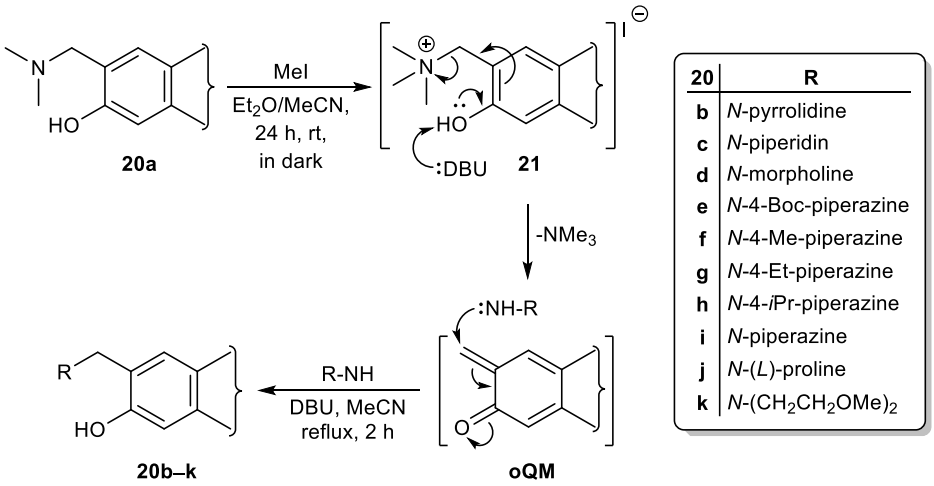
3.11. Based on the *in vitro* pharmacological studies, the substitution of the aromatic benzene ring proved to be detrimental once again, as it either caused lower selectivity and/or worse antiproliferative effect. The chalcones (somewhat unexpectedly) proved to be ineffective, and only flavones **18a**–**c** were found to exhibit a selective and strong anticancer effect.

3.12. To explore the usefulness of multicomponent reactions, firstly estrone (**E1**) was subjected to *Mannich* reaction with dimethylamine, which rapidly and selectively furnished the desired 2-aminomethylated compound (**20a**, 80%, *Scheme 10*), regardless of the formaldehyde source used. However, in the case of cyclic amines, much longer reaction times were necessary to achieve full conversion, and separation of the regioisomers was difficult.



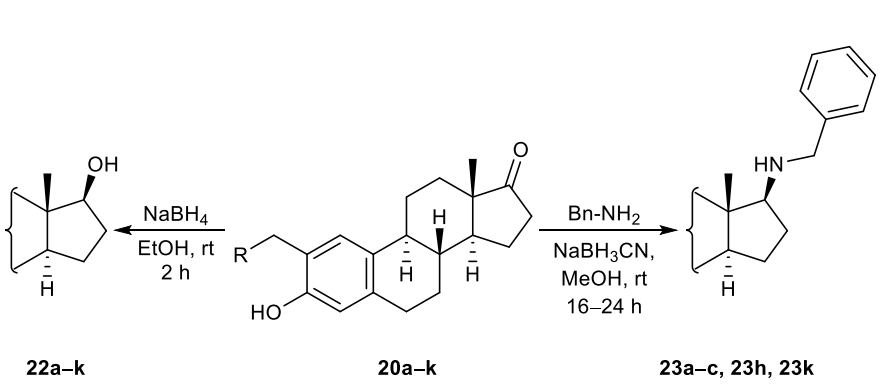
Scheme 10

3.13. As an alternative synthetic method, the *aza-Michael* addition of amines to *in situ* formed *ortho*-quinone methides (oQM) was identified as a viable strategy, since the latter can easily be obtained by performing *Hofmann* elimination on the quaternary salt obtained from **20a**. Quaternization proceeded nearly quantitatively with methyl iodide, however the reaction must be performed in the dark because the salt **21** is susceptible to photocatalytic degradation. Based on the model reaction with pyrrolidine, 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) was found to be the ideal base, and the reaction was repeated with numerous other secondary amines. As a result, the desired 2-aminomethylated estrone derivatives (**20b**–**k**, *Scheme 11*) were obtained in moderate to excellent yields (62–89%).



Scheme 11

3.14. Since the 17-keto group of the sterane core was left untouched, we expanded the scope of our investigations by modifying it. Reduction with sodium borohydride gave the corresponding secondary alcohols (**22a**–**k**, 66–91%), while the *Borch* reductive amination with benzylamine gave the desired 17-benzyl derivatives (**23a**–**c**, **23h**, **23k**, 56–71%, *Scheme 12*). The latter transformation was performed only in five cases, since we expected these compounds to have pronounced anticancer properties, based on the results of *in silico* modelling of certain pharmacological properties.



20, 22, 23	R
a	*N*-Me_2
b	*N*-pyrrolidine
c	*N*-piperidine
d	*N*-morpholine
e	*N*-4-Boc-piperazine
f	*N*-4-Me-piperazine
g	*N*-4-Et-piperazine
h	*N*-4-*i*Pr-piperazine
i	*N*-piperazine
j	*N*-(*L*)-proline
k	*N*-$(CH_2CH_2OMe)_2$

Scheme 12

3.15. The *in vitro* pharmacological studies showed that compounds containing dimethyl (**a**), pyrrolidine (**b)**, *tert*-butoxycarbonyl protected piperazine (**e**) or dimethoxyethane (**k**) side chains have performed the best results. The 17-keto (**20a**–**k**) and 17-OH (**22a**–**k**) derivatives sometimes showed remarkably different behaviours, which we cannot explain with a clear structure-activity relationship. All of the 17-benzylated compounds (**23a**–**c**, **23h**, **23k**) had significantly lower IC_{50} values, but their selectivity was completely lost, presumably due to their increased lipophilicity.

4. Scientific publications directly related to the Ph.D. Thesis

1. **Barnabás Molnár**, Mohana Krishna Gopisetty, Dóra Izabella Adamecz, Mónika Kiricsi, Éva Frank
   Multistep synthesis and *in vitro* anticancer evaluation of 2-pyrazolyl-estradiol derivatives, pyrazolocoumarin-estradiol hybrids and analogous compounds
   Molecules, **2020**, *25*, 4039.

   IF = 4.412

2. **Barnabás Molnár**, Mohana K. Gopisetty, Ferenc István Nagy, Dóra Izabella Adamecz, Zsolt Kása, Mónika Kiricsi, Éva Frank
   Efficient access to domain-integrated estradiol-flavone hybrids *via* the corresponding chalcones and their in vitro anticancer potential
   Steroids, **2022**, *187*, 109099.

   IF = 2.760*

3. **Barnabás Molnár**, Njangiru Isaac Kinyua, Gergő Mótyán, Péter Leits, István Zupkó, Renáta Minorics, György T. Balogh, Éva Frank
   Regioselective synthesis, physicochemical properties and anticancer activity of 2-aminomethylated estrone derivatives
   The Journal of Steroid Biochemistry and Molecular Biology, **2022**, *219*, 106064.

   IF = 5.011*

Total IF = 12.183*

MTMT identifier: 10065235

5. Other scientific publications

1. Gergő Mótyán, **Barnabás Molnár**, János Wölfling, Éva Frank
   Microwave-assisted stereoselective heterocyclization to novel ring D-fused arylpyrazolines in the estrone series
   Molecules, **2019**, *24*, 569.

   IF = 3.267

2. Anita Kiss, Bianka Edina Herman, Tamás Görbe, Erzsébet Mernyák, **Barnabás Molnár**, János Wölfling, Mihály Szécsi, Gyula Schneider
   Synthesis of novel 17-triazolyl-androst-5-en-3-ol epimers *via* Cu(I)-catalyzed azide-alkyne cycloaddition and their inhibitory effect on 17α-hydroxylase/C17,20-lyase
   Steroids, **2018**, *135*, 79.

IF = 2.136

Total IF = 5.403

6. Lectures and posters related to the Ph.D. Thesis

1. **Barnabás Molnár**, Éva Frank
   2-Pirazolil-ösztradiol származékok, pirazolokumarin-ösztradiol hibridek és analóg modellvegyületeik előállítása
   MTA Szteroid- és Terpenoidkémiai Munkabizottsági ülése, Szeged, Hungary, 22 November 2019.
2. **Barnabás Molnár**, Éva Frank
   A-gyűrűn módosított ösztradiol származékok szintézise
   Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány előadóülése, online, 10 May 2020.
3. **Barnabás Molnár**, Éva Frank
   A-gyűrűn módosított ösztránvázas szteroidok: aminometil-, kalkon- és flavon-származékok szintézise
   Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány előadóülése, online, 25 May 2021.
4. **Barnabás Molnár**, Éva Frank
   2-Szubsztituált ösztránvázas vegyületek előállítása és továbbalakítása
   MTA Szteroid- és Terpenoidkémiai Munkabizottság ülése, online, 6 December 2021.

7. Other lectures and posters

1. **Barnabás Molnár**, Gergő Mótyán, Éva Frank
   Ösztránváz D-gyűrűjéhez kondenzált pirazolinok mikrohullámú szintézise
   Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány előadóülése, Szeged, Hungary, 12 May 2016.
2. **Barnabás Molnár**, Gergő Mótyán, Éva Frank
   Microwave-assisted synthesis of ring D-condensed pyrazolines in the estrone series
   1st Hungarian-Norwegian Summer School on Bioactive Substance Research, Tromsø, Norway, 11–26 July 2016.
3. **Barnabás Molnár**, Gergő Mótyán, Éva Frank
   Pirazolin-gyűrűvel módosított ösztron származékok előállítása mikrohullámú aktiválással
   XXXIII. Országos Tudományos Diákköri Konferencia, Miskolc, Hungary
   29–31 March 2017.
4. **Barnabás Molnár**, Gergő Mótyán, Ádám Baji, Éva Frank
   Pirazolin- és triazolgyűrűvel módosított ösztron származékok előállítása mikrohullámú aktiválással
   MKE Vegyészkonferencia, Hajdúszoboszló, Hungary, 19–21 June, 2017 (poster presentation).
5. **Barnabás Molnár**, Gergő Mótyán, Ádám Baji, Éva Frank
   Ösztránváz D-gyűrűjéhez kondenzált *N*-heterociklusok előállítása
   MTA Szteroid- és Terpenoidkémiai Munkabizottsági ülése, Szeged, Hungary, 27 November, 2017.
6. **Barnabás Molnár**, Gergő Mótyán, Ádám Baji, Éva Frank
   Ösztránvázhoz kondenzált triazolok és analóg vegyületek szintézise
   Szegedi Ifjú Szerves Kémikusok Támogatásáért Alapítvány előadóülése, Szeged, Hungary, 15 May 2018.

7. **Barnabás Molnár**, Gergő Mótyán, Mohana K. Gopisetty, Mónika Kiricsi, Małgorzata A. Marć, Éva A. Enyedy, Éva Frank
   Microwave-assisted synthesis of steroidal 5-aminopyrazoles and the evaluation of their cytotoxic effect *in vitro*
   2nd Euro Chemistry Conference, Valencia, Spain, 17–19 June 2019 (poster presentation).

Cumulative IF = 17.586*