Synthesis, *in vitro* steroid receptor binding and *in silico* testing of novel 17α-(pyridin-2-yl)estra-1,3,5(10),16-tetraen derivatives

Milica Stevanović [1], Sofija Bekić [1], Anđelka Ćelić [2], Ivana Kuzminac [1]

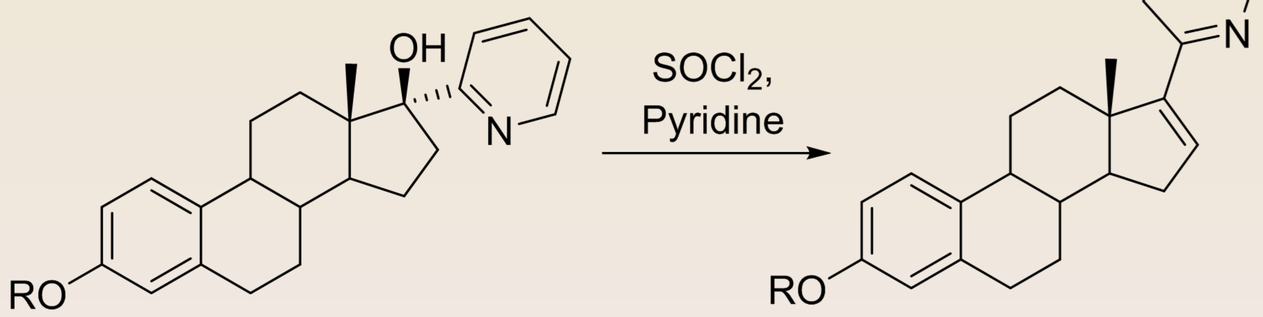
[1] University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, Novi Sad, Serbia

[2] University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, Novi Sad, Serbia

milica.stevanovic@dh.uns.ac.rs

Introduction: Steroid compounds incessantly attract attention due to their biological and clinical importance. Considering that many cancers are hormone-dependent, synthetically modified steroid molecules are suitable candidates for the treatment of these malignancies. It has been established that steroid compounds with a heterocyclic ring or heteroatoms in their structure represent potential anticancer agents, and also have other important pharmacological properties

Scheme 1.



1 R=H
2 R=Me
3 R=Bn

4 R=H
5 R=Me
6 R=Bn

Table 1. Calculated molecular properties

Comp.	MF	MW	HBA	HBD	LogP	nrotb	TPSA	MR	No.ring
4	$C_{23}H_{25}NO$	331.45	2	1	4.56	1	33.12	102.47	5
5	$C_{24}H_{27}NO$	345.48	2	0	4.95	2	22.12	106.93	5
6	$C_{30}H_{31}NO$	421.57	2	0	6.15	4	22.12	131.42	6

MW, molecular weight (gmol-1; <500); logP, logarithm of compound partition coefficient between n-octanol and water (<5); HBA, number of hydrogen bond acceptors (<10); HBD, number of hydrogen bond donors (<5); MR, molar refractivity; TPSA, topological polar surface area (Å^2; <140); nrotb, number of rotatable bonds (<12).

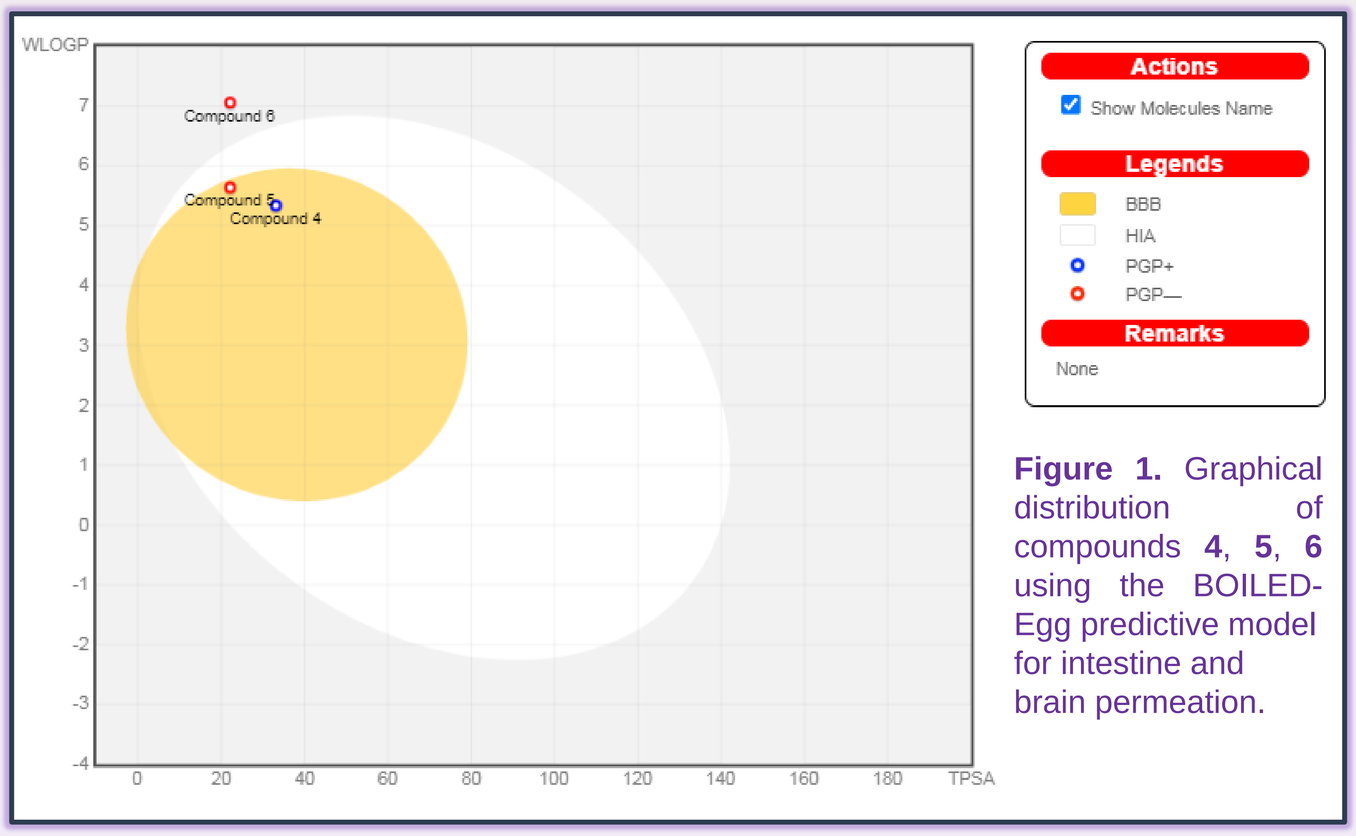


Figure 1. Graphical distribution of compounds **4**, **5**, **6** using the BOILED-Egg predictive model for intestine and brain permeation.

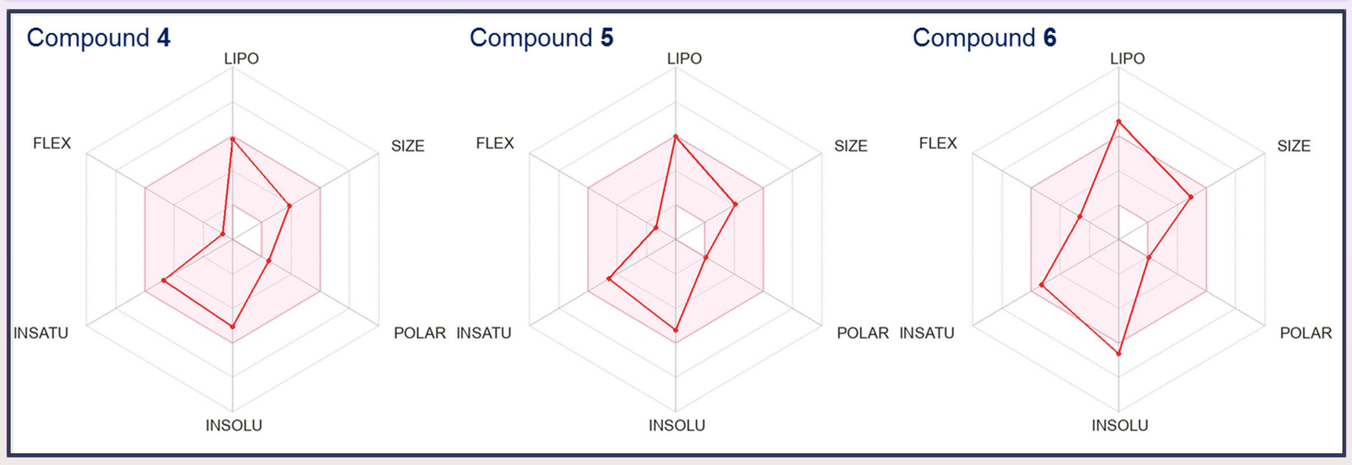


Figure 2. The Bioavailability Radar of synthesized compounds enables faster insight into the drug-likeness of compounds. The pink area represents the optimal range for lipophilicity, size, polarity, solubility, saturation, and flexibility. All of synthesized compounds falls entirely or partially in the pink area which represents the optimal range for each properties.

Results and discussion: We have synthesized novel 17-(pyridin-2-yl)estra-1,3,5(10),16-tetraen compounds, starting from 17β-hydoxy-17α-(pyridin-2-yl)-estra-1,3,5(10)-triene derivatives by dehydratation reactions. For all synthesized compounds we analyzed *in silico* ADMET properties using the online SwissADME tool and ProToxII virtual lab. Since these modified compounds are based on steroidal scaffolds, their relative binding affinities for the ligand-binding domains of estrogen receptor α and β and androgen receptor were evaluated using a fluorescent assay in yeast.

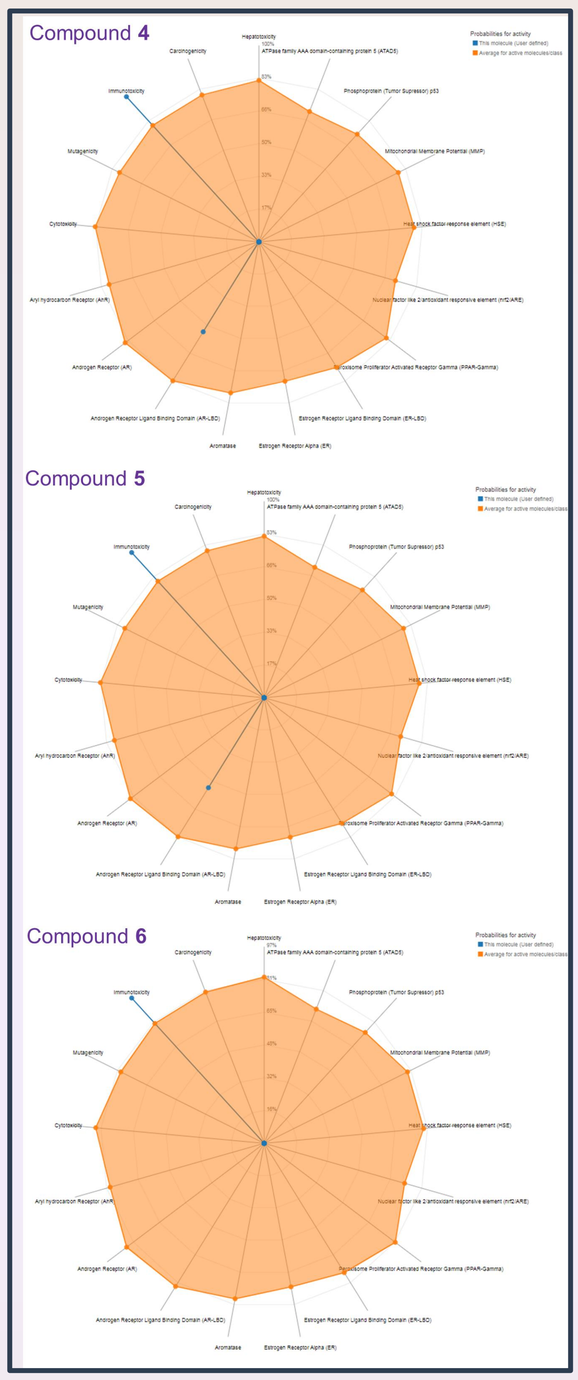
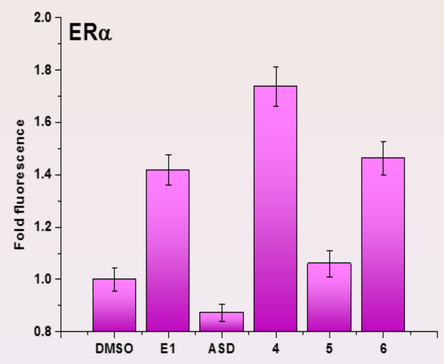
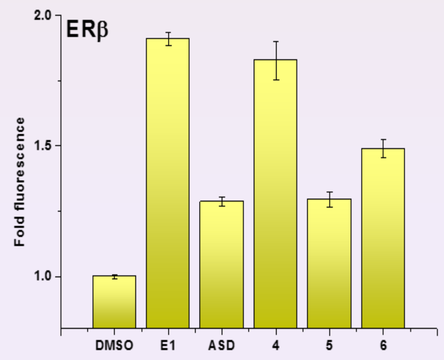


Figure 3. The Radar Toxicity Table provides a quick illustration of the positive toxicity results compared to the average of its class.





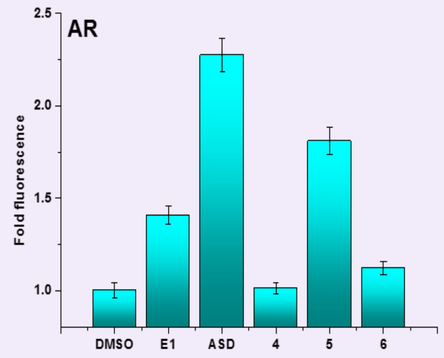


Figure 4. Relative binding affinities of tested steroid derivatives expressed as fold fluorescence change between ligand-treated and control cells in the absence of ligand expressing ERα LBD-YFP, ERβ LBD-YFP and AR LBD-YFP; control ligands: E1-estrone, ASD-androstenedione. Yeast strains expressing ERα LBD-YFP, ERβ LBD-YFP or AR LBD-YFP were tested and measured by fluorimetry in 96-well format. Fold fluorescence changes were calculated after 14-16 hours of exposure to 10 μM steroid, and are directly proportional to relative binding affinity. Yeast cells expressing ERα LBD-YFP or ERβ LBD-YFP were treated with estrone (E1) as a high-affinity ligand (+ control) and androstenedione (ASD) as a ligand with low-affinity (- control).

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Conclusion: In this work, we have synthesized novel 17-(pyridin-2-yl)estra-1,3,5(10),16-tetraen compounds **4-6** (Schema 1) by dehydration reactions. Based on the BOILED-Egg model it can be concluded that compounds **4** and **5** are capable of both modes of absorption, while compound **6** is not predicted to be absorbed in these ways (Figure 1). The Bioavailability radar showed a small deviation from the pink area for compound **6**, while other compounds don't show any deviation (Figure 2). The toxicity radar charts indicate possible immunotoxicity for all three synthesized compounds (Figure 3). As shown in Figure 4, compounds **4** and **6** showed binding affinity to both ER isoforms compared to natural ligand, estrone (E1).