Chapitre 6

Conception, synthèse et évaluation de l'activité inhibitrice de trois séries de dérivés estranes à l'encontre du cytochrome P450 (CYP) 1B1

Avant-propos

Le chapitre 6 inclut un manuscrit qui nécessite encore l'ajout de certains résultats basés sur des essais biologiques en cours. La rédaction de cet article est néanmoins presque entièrement complétée et ce manuscrit sera donc bientôt soumis par mon directeur de recherche.

De nombreux auteurs ont également participé à cette section de mon projet de maîtrise. Jenny Roy a réalisé tous les essais biologiques présentés dans ce papier, René Maltais m'a apporté son aide pour le design et la synthèse des composés ainsi que pour la mise en forme de certaines figures de l'article. Francisco Cortés-Benítez a effectué plusieurs études de "docking" et a participé de façon importante à la conception des inhibiteurs stéroïdiens de la CYP1B1.

Mon directeur de recherche, Donald Poirier, s'est occupé de la supervision du projet en émettant plusieurs suggestions pour le design et la synthèse des composés présentés dans cet article mais aussi pour la mise en place d'essais biologiques justifiant leur utilisation thérapeutique. Il a également contribué de façon importante à l'amélioration du manuscrit.

Pour ma part, j'ai réalisé la synthèse, la purification et la caractérisation de tous les nouveaux composés présentés dans ce manuscrit. J'ai également rédigé la majorité du contenu scientifique présenté dans cet article incluant la partie expérimentale.

Résumé

L’enzyme cytochrome P450 (CYP) 1B1 est une cible thérapeutique intéressante car elle est impliquée dans la bioactivation des procarcinogènes et la pharmacorésistance; de plus, cette enzyme est surexprimée dans plusieurs cas de cancer. L’utilisation d’un inhibiteur de la CYP1B1 pourrait donc être une stratégie prometteuse dans un contexte de multithérapies pour le traitement de certains cancers. Basé sur des études de "docking" et sur des relations structure-activité, nous avons réalisé la synthèse de trois séries de dérivés C18-stéroïdiens : 12 dérivés de l’estrone (E1) et de l’estradiol (E2) portant un noyau 3- ou 4-pyridine en C2, C3 ou C4 du noyau stéroïdien (**Série 1**), huit dérivés avec différents groupes soufrés en C3 (**Série 2**) et 19 dérivés de E1 et E2 portant différents groupes aryles en C2 (**Série 3**). L'activité inhibitrice de la CYP1B1 par ces trois séries de dérivés stéroïdiens a été évaluée via le test éthoxyrésorufine-*O*-dééthylase (EROD) et comparée à celle de l'α-naphthoflavone (ANF), un inhibiteur non stéroïdien connu de la CYP1B1. Pour chaque série, nous avons observé que les dérivés E2 (17β-OH) étaient plus actifs que leurs analogues oxydés en C17 (dérivés E1) mettant en évidence le rôle clé du 17β-OH dans l'interaction avec la CYP1B1. Les résultats les plus intéressants ont été obtenus avec la **Série 3**, en effet, presque tous les composés de cette série étaient de puissants inhibiteurs de la CYP1B1. Parmi cette série, le composé **20b**, avec un goupe 4-méthoxyphényle en C2, est particulièrement intéressant car il est plus puissant que l'ANF et parce que les groupes méthoxyles sont connus pour jouer un rôle dans la sélectivité pour la CYP1B1. Nous avons également développé quatre dérivés du composé **20b** afin d'améliorer sa stabilité métabolique et afin d'apprécier l'impact de ces modifications chimiques sur l'activité inhibitrice de la CYP1B1.

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Design, synthesis and evaluation of cytochrome P450 (CYP) 1B1 inhibitory activity of three series of estrane derivatives

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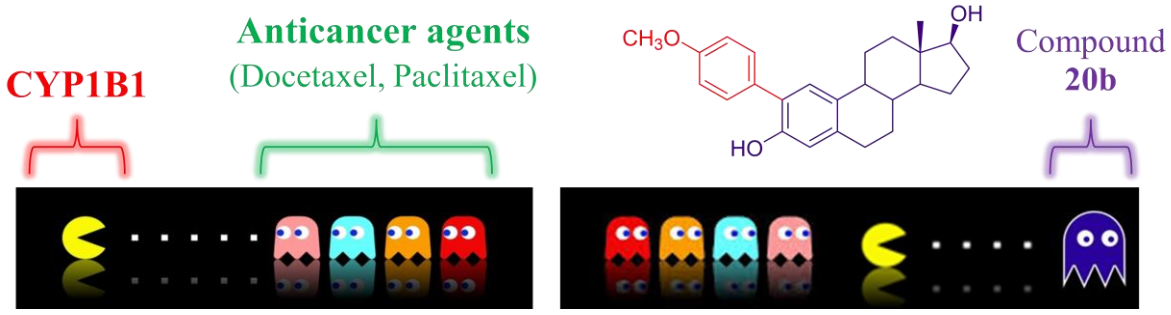
Abstract

Cytochrome P450 (CYP) 1B1 is an attractive therapeutic target because it is involved in the bioactivation of procarcinogens and drug-resistance, and this enzyme is overexpressed in several types of cancer. Therefore, a CYP1B1 inhibitor could be a promising strategy within a context of multitherapies for the treatment of some cancers. Based on docking and structure-activity relationship studies, we performed the synthesis of three series of estrane derivatives : twelve estrone (E1) and 17β-estradiol (E2) derivatives bearing a 3- or a 4-pyridine core at C2, C3 or C4 of the steroid nucleus (**Series 1**), eight estrane derivatives with different sulfur groups at postion 3 (**Series 2**) and nineteen E1 and E2 derivatives bearing distinct aryl moieties at C2 (**Series 3**). The CYP1B1 inhibitory activity of these three series was evaluated using the ethoxyresorufin-*O*-deethylase (EROD) assay and compared to that of α-naphthoflavone (ANF), a known non-steroidal CYP1B1 inhibitor. For each series, we observed that the E2 derivatives (17β-OH) were more active than their oxidized analogs at C17 (E1 derivatives) highlighting the key role of the 17β-OH for the interaction with CYP1B1. The most potent inhibitors were obtained with **Series 3**. Among these compounds, the 2-(*p*-methoxyphenyl)-E2 (**20b**) is particulary interesting because it was more potent than ANF and because methoxy groups are known to play a role in the selectivity for CYP1B1 towards CYP1A1 and CYP1A2. We also developed four D-ring derivatives of compound **20b** in order to improve its metabolic stability and to appreciate the impact of these chemical modifications on CYP1B1 inhibitory activity.



INTRODUCTION

Cytochromes P450 (CYPs) constitute an essential family of hemoproteins involved in the metabolism of a wide variety of endogenic and xenobiotic compounds [1-4]. The CYP1 family belongs to the eighteen CYP gene families known in humans and includes three enzymes : CYP1A1, CYP1A2 and CYP1B1 [3, 4]. CYP1B1 is an attractive therapeutic target for different reasons : 1) it catalyzes the 4-hydroxylation of 17β-estradiol (E2) leading subsequently to the formation of E2-3,4-quinone, a mutagenic compound able to bind DNA covalently; 2) it is involved in the bioactivation of several procarcinogens such as benzo[α]pyrene and 3) it is associated with drug-resistance by interacting with the metabolism of some anticancer agents, such as docetaxel, paclitaxel and cisplatine [5-11]. Furthermore, an overexpression of CYP1B1 has been observed in distinct types of human cancers (breast, lung, esophagus, skin, testis, colon, lymph node and brain). Therefore, a CYP1B1 inhibitor associated with an anticancer agent could be a promising strategy to treat cases of cancer in which CYP1B1 is overexpressed and thus developed drug resistance [12, 13].

In a review recently published [14], we reported the different CYP1B1 inhibitors identified since 2003 among distinct families of chemicals : flavonoids, *trans*-stilbenes, coumarins, alkaloids, anthraquinones and several other compounds. Flavonoid and stilbene derivatives have been extensively studied to interact with CYP1 enzymes and the best CYP1B1 inhibitors known to date belong to these two families of chemicals [13-16]. Surprisingly, only two studies were conducted on CYP1B1 inhibition by steroid derivatives [17, 18]. We therefore decided to focus on this family of narural products because our research group is specialized in the synthesis of steroid derivatives (C18-, C19- and C21-steroids) to interact with the steroidogenesis enzymes [19-24]. The inhibitory activity of CYP1B1 by 90 steroid derivatives was thus evaluated using the ethoxyresorufin-*O*-deethylase (EROD) assay. This screening led to the identification of 3-thioestrone (IC_{50} = 3.4 μM) as the most potent CYP1B1 inhibitor among this series of steroid derivatives [25]. In this regard, we observed by molecular modeling studies that the 3-SH of 3-thioestrone is closer (3.36 Å) to the iron atom of the CYP1B1 heme system than the 3-OH of estrone (E1) and E2. These observations also suggest that the CYP1B1 inhibitory activity could be improve by introducing a chemical group, such as a pyridinyl, known to interact with heme systems on the A-ring of the C18-steroid core.

Otherwise, it should be emphasized that an estrane nucleus with a phenyl ring at C2 shares some structural similarity with that of α-naphthoflavone (ANF) (**Figures 1A** and **1B**), a known potent CYP1B1 inhibitor that was co-crystallized with CYP1B1 [26]. In this structure, the phenyl moiety of ANF is oriented towards the iron atom of the heme system of CYP1B1. By introducing different chemical groups on the phenyl moiety of ANF, Cui *et al.* have identified the most potent CYP1B1 inhibitor to date (3'-F-6,7,10-tri-MeO-ANF, **Figure 1A**) [11]. Interestingly, the tricyclic core of this ANF derivative can be superimposed with the A/B/C-rings of the steroidal scaffold (**Figure 1**).

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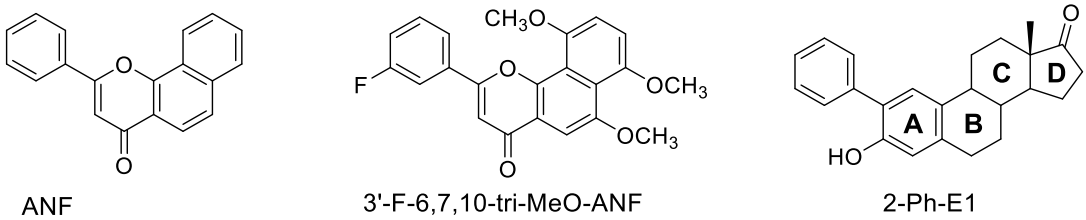

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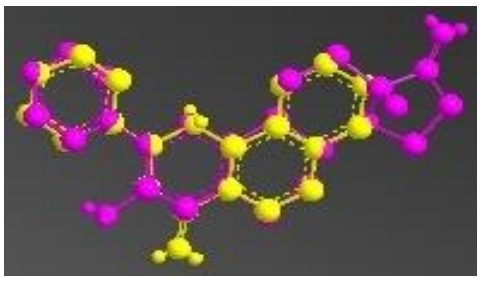

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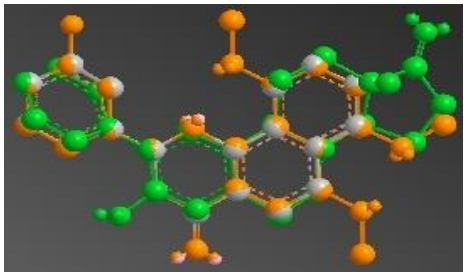


Figure 1. (**A**) Chemical structures of α-naphthoflavone (ANF), 3'-F-6,7,10-tri-MeO-ANF and 2-Ph-E1, (**B**) superimposition of ANF (*yellow*) and 2-Ph-E1 (*purple*) cores, and (**C**) superimposition of ANF (*grey*), 3'-F-6,7,10-tri-MeO-ANF (*orange*) and 2-Ph-E1 (*green*) cores. Steroid A-D rings are identified within the chemical structure of 2-Ph-E1. Pictures were produced using ChemBio3D Ultra software. Energy minimization was performed using MM2 and the structures were superimposed using the overlay option.

Based on these observations and on docking studies, we synthesized three series of estrane derivatives in order to identify potent CYP1B1 inhibitors (**Figure 2**). The first series regroups twelve E1 and E2 derivatives bearing a 3- or a 4-pyridinyl moiety at C2, C3 or C4 of the estrane core (Series 1, **Figure 2**) [27]. Indeed, the nitrogen atom of pyridine is known to generate interactions with the iron of heme systems [28]. The results obtained with this series of pyridine-estrane derivatives have been the subject of a preliminary report by our research group [27]. Briefly, it was observed that estrane derivatives bearing the pyridine moiety at C2 are much more potent CYP1B1 inhibitors than those with a pyridine ring at C3 or C4. The

best two CYP1B1 inhibitors of Series 1 were the E2-derivatives with a 3-pyridinyl at C2 (compound **1b**) and its counterpart with a 4-pyridinyl at C2 (compound **2b**) (IC_{50} = 0.011 and 0.032 μM, respectively).

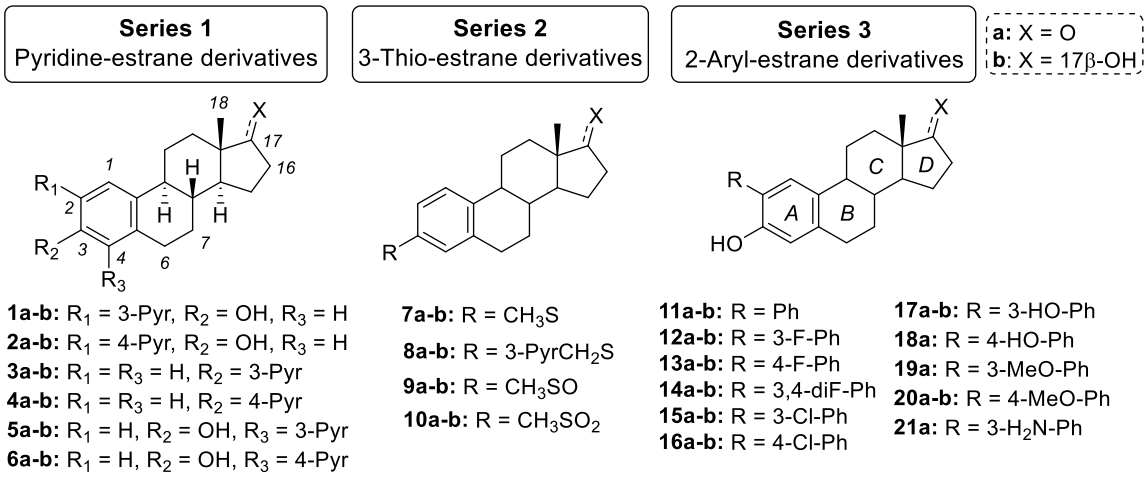


Figure 2. Chemical structures of the three series of estrane derivatives designed and synthesized for the inhibition of CYP1B1. Partial numbering of carbons (left structure) and steroid (A-D) ring identification (right structure) are reported.

Herein, we report the chemical synthesis, characterization and CYP1B1 inhibition (EROD test) of additional estrane derivatives represented by Series 2 and 3 (**Figure 2**). In the second series, a sulfur group was introduced at C3 to promote an interaction with the heme of porphyrin. In the third series, the design of E1 and E2 derivatives was based on the similarity of the estrane nucleus with ANF and 3'-F-6,7,10-tri-MeO-ANF. Finally, we generated four D-ring derivatives of our best CYP1B1 inhibitor, compound **20b**, to reduce metabolization of the 17β-OH group.

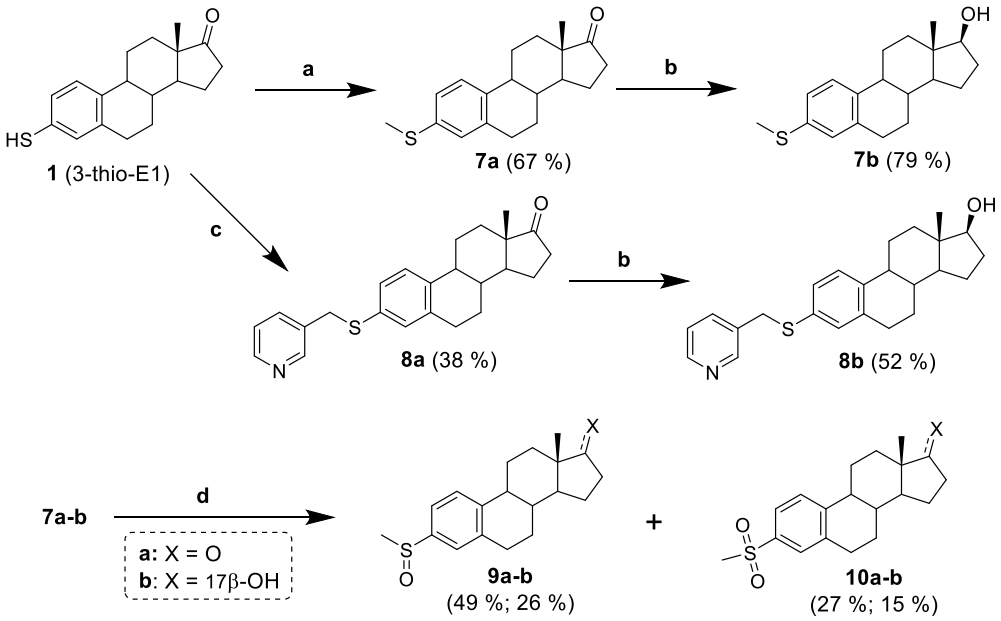
RESULTS AND DISCUSSION

1. Synthesis of new estrane derivatives

1.1. Synthesis of estrane-sulfur derivatives (Series 2)

Based on the results obtained with 3-thio-E1 as CYP1B1 inhibitor [25], and on docking studies performed with this steroid derivative, we synthesized eight estrane derivatives bearing different sulfur groups at C3 (compounds **7a-b** to **10a-b**, **Scheme 1**).

Compound **7a**, with a thiomethyl at C3, was obtained in one step from 3-thio-E1 by a methylation with methyl iodide in presence of cesium carbonate (Cs_2CO_3). Thereafter, compound **7b** was obtained from **7a** by a reduction of the C17 ketone with sodium borohydride ($NaBH_4$).



Scheme 1. Chemical synthesis of 3-thio-estrane derivatives (Series 2). Reagents and conditions: (**a**) CH_3I, Cs_2CO_3, ACN, reflux, 2 h; (**b**) $NaBH_4$, MeOH-DCM (9:1), 0 °C, 2 h; (**c**) 3-(bromomethyl)pyridine hydrobromide, Cs_2CO_3, TEA, TBAI, ACN, reflux, overnight; (**d**) Oxone, MeOH-H_2O (8:2), 0 °C, 2 h.

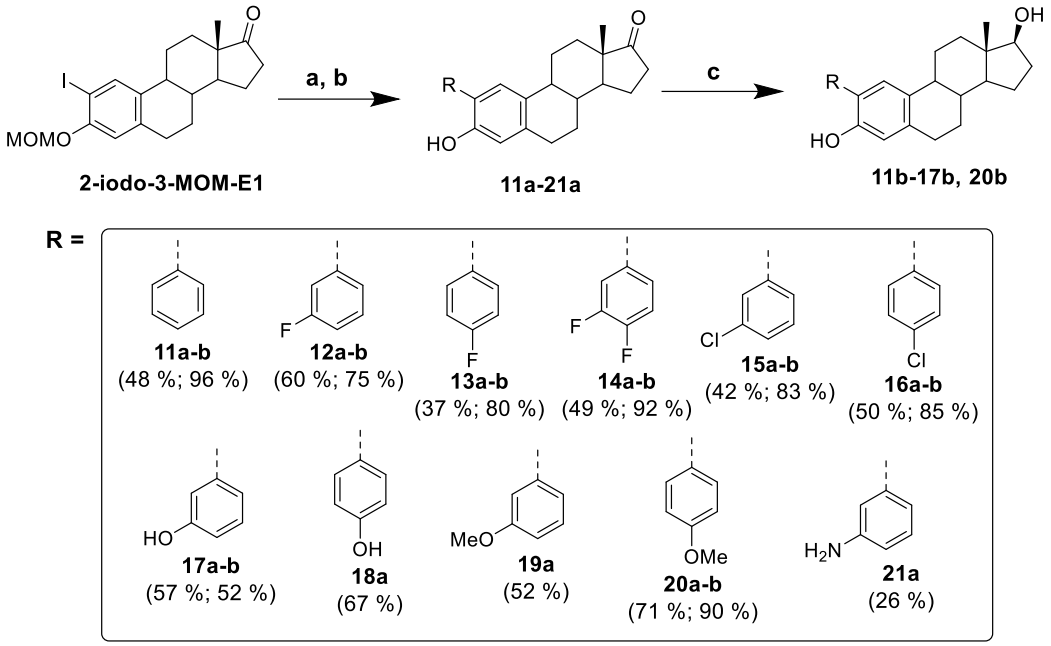
Compounds **8a-b** are the E1 and E2 derivatives, respectively, with a pyridin-3-ylmethanethio group at C3 of the steroid core. Since pyridine is known to generate interactions with heme sytems [27, 28], we synthesized these compounds to possibly benefit from a combined effect of the sulfur atom and the pyridine moiety. Compound **8a** was obtained by reacting 3-thio-E1 with 3-(bromomethyl)pyridine hydrobromide in refluxing acetonitrile (ACN), with Cs_2CO_3 and triethylamine (TEA) as bases. A small amount of tetrabutylammonium iodide (TBAI) was also added to promote the reaction by replacing the bromine atom of 3-(bromomethyl)pyridine by a more reactive iodine atom. Compound **8b** was next obtained by a reduction of **8a** with $NaBH_4$.

Finally, we synthesized the sulfoxides **9a-b** and the sulfones **10a-b** because they are highly probable metabolites of **7a-b** due to the oxidizability of sulphur atom. Compounds **9a**

and **10a** were obtained in the same time from **7a** using potassium peroxymonosulfate (Oxone) as oxidizing agent. This reaction was performed in MeOH-H_2O at 0 °C and must be carefully monitored to avoid complete oxidation of **7a** to **10a**. Compounds **9b** and **10b** were obtained in the same conditions used for the synthesis of **9a** and **10a**, but using **7b** as starting product.

1.2. Synthesis of 2-aryl-estrane derivatives (Series 3)

Based on the structural similarity between the steroid nucleus and ANF as well as on the good results obtained by Cui *et al.* [11] by introducing several small groups to the phenyl moiety of ANF, we developed a third series of estrane derivatives having different aryl groups at C2 of the steroid core (compounds **11a-b** to **21a**, **Scheme 2**). Phenyl groups bearing small chemical functions were selected because a too large group could impair the insertion of the steroid derivative into the catalytic site of CYP1B1. We chose phenyl groups with a fluorine atom (**12a-b**, **13a-b** and **14a-b**) or a chlorine atom (**15a-b** and **16a-b**) because ANF derivatives bearing halogens on their phenyl core were very potent CYP1B1 inhibitors [11]. Phenol and anisole derivatives **17a-b**, **18a**, **19a** and **20a-b** were also prepared because it was observed that the presence of hydroxyl or methoxy groups can modulate the selectivity of inhibition towards CYP1B1 [13, 16]. The aniline derivative **21a** was also prepared based on the ability of the nitrogen atom to interact with the iron of heme groups. Aryl derivatives bearing the same substituent but at different positions (3- and 4-) were thus tested to complement our SAR study. Finally, 2-phenyl-estrane derivatives **11a-b** were selected as references for this series of 2-aryl-estrane derivatives.



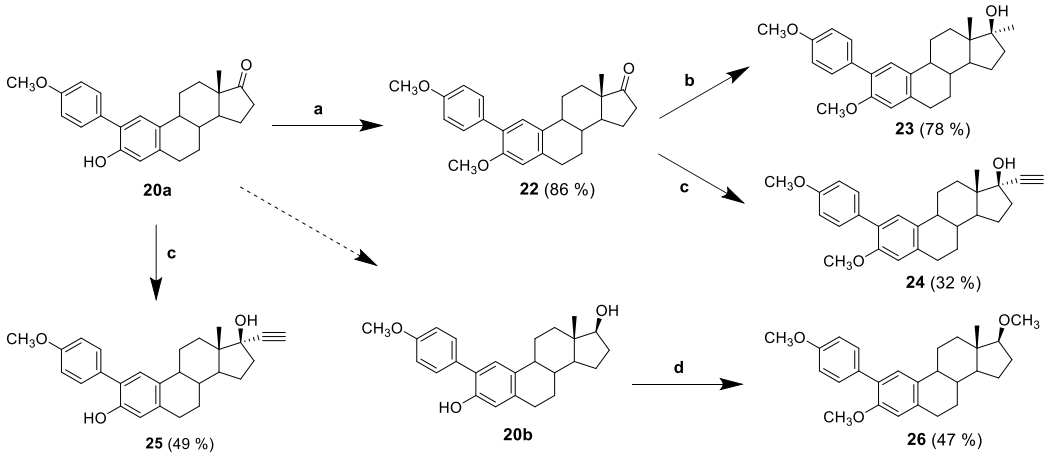
Scheme 2. Chemical synthesis of the 2-aryl-estrane derivatives (Series 3). Reagents and conditions: (**a**) R-B(OH)$_2$, Pd(dppf)Cl$_2$, K$_3$PO$_4$, DMF, MW, 120 °C, 3-4 h; (**b**) 10 % HCl aq.-MeOH (1:9), 50 °C, overnight; (**c**) NaBH$_4$, MeOH-DCM (9:1), 0 °C, 2 h.

The nineteen estrane derivatives of the Series 3 (compounds **11a-b** to **21a**) were synthesized by reacting 2-iodo-3-methoxymethylether(MOM)-E1 with the corresponding arylboronic acid in the same reaction conditions used for the synthesis of the series of pyridine-estrane derivatives (Series 1). Reactions were performed at 120 °C in dimethylformamide (DMF) under microwave irradiation with potassium phosphate tribasic (K$_3$PO$_4$) as base and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(dppf)Cl$_2$) as catalyst. This Suzuki coupling reaction provided these 2-aryl-estrane derivatives in good yields, but the introduction of the MOM protecting group at C3 of the steroid backbone is important. In fact, this ether group probably promotes the reaction by complexing the palladium catalyst. After the Suzuki coupling, the MOM group was hydrolysed using chlorhydric acid in methanol to obtain the eleven 2-aryl-E1 derivatives **11a-21a**. Based on their CYP1B1 inhibitory activities, eight candidates were selected and the C17-carbonyl was reduced with NaBH$_4$ to obtain the 2-aryl-E2 derivatives **11b-17b** and **20b**.

*1.3. Synthesis of D-ring derivatives of **20b** (compounds **23-26**)*

In our study carried out with pyridine-estrane derivatives (Series 1) [23], we evaluated the plasmatic concentration of **1b** (**Figure 2**) in rats. We observed that the alcohol **1b** was oxidized at C17 to form the ketone **1a**, and that both compounds **1a-b** were eliminated after 6

h. To stabilize the 17β-OH functionality toward an oxidation by Phase-I metabolism enzymes as well as through a glucuronidation or a sulfatation by Phase-II metabolism enzymes, we synthesized four D-ring derivatives. The potent CYP1B1 inhibitor **20b** (Series 3) was thus selected and modified by adding different group known to protect a 17β-OH group.



Scheme 3. Synthesis of D-ring derivatives of **20b**. Reagents and conditions**:** (**a**) CH_3I, Cs_2CO_3, ACN, reflux, 2-3 h; (**b**) CH_3MgI, toluene, 80 °C (4 h) to rt (overnight); (**c**) *i.* TMS-acetylene, MeLi, THF, rt, overnight; *ii.* K_2CO_3, MeOH, rt, 5 h; (**d**) CH_3I, NaH, DMF, rt, overnight.

The intermediate compound **22** was synthesized from **20a** by a methylation of the 3-OH group with methyl iodide and Cs_2CO_3 in refluxing ACN and next used in the preparation of **23** and **24**. Compound **23** was obtained by adding a methyl group at position 17α of **22** using methylmagnesium iodide in anhydrous toluene. Compound **24** was obtained from **22** through an ethynylation at position 17α in two steps: 1) adding lithium trimethylsilylacetylide in anhydrous tetrahydrofuran (THF) and 2) hydrolyzing the silylacetylenic intermediate. Compound **25** was obtained using the same reaction conditions reported for the synthesis of **24**, but with **20a** as starting product. This compound was prepared in order to evaluate the impact of the methoxy group at C3 on the metabolic stability of these D-ring derivatives. Finally, compound **26** was synthesized from **20b** by a dimethylation of its 3- and 17β-OH groups with methyl iodide in anhydrous DMF and sodium hydride as strong base.

2. Assesment of estrane derivatives as CYP1B1 inhibitors

The inhibitory activities towards CYP1B1 of these newly synthesized estrane derivatives (Series 2 and 3; **Figure 2**) were evaluated using the standard EROD assay which

is conventionally used to assess CYP1 activity (**Tables 1** and **2**). These assays were performed with recombinant human CYP1B1 enzyme and a NADPH regenerating system. The transformation of resorufin ethyl ether by CYP1B1 into fluorescent resorufin was measured in this enzymatic assay to evaluate the CYP1B1 activity. ANF, a known potent CYP1B1 inhibitor, was used as reference for these EROD assays [15, 26].

2.1. CYP1B1 inhibitory activities of estrane-sulfur derivatives

In regard of the EROD assay results obtained with the eight thio-estrane derivatives as CYP1B1 inhibitors (**Table 1**), only **8a-b** have shown a significant inhibitory activity against CYP1B1. These two compounds bear the same pyridin-3-ylmethanethio group at C3 and the alcohol **8b** (17β-OH) is more active than its oxidized homolog at C17, the ketone **8a**. This observation highlights the key role of the 17β-OH for the CYP1B1 inhibition and is in accord with the results ontained with compounds of Series 1 [27]. On the other hand, compounds **7a-b**, with a thiomethyl group at C3 and its likely metabolites resulting from the oxidation of the sulfur atom, compounds **9a-b** and **10a-b**, are very weak inhibitors. From these results, the sulfur atom does not seem to have a major role for the inhibition of CYP1B1. However, adding a pyridine moiety to the steroid core led to a significant gain in inhibitory activity as exemplified with compounds **8a-b**. They are however less potent inhibitors than ANF (47.7 ± 2.8, 60.7 ± 2.8 and 94.0 ± 3.2 % of inhibition at 0.3 μM for **8a, 8b** and ANF, respectively).

Table 1. Inhibition of CYP1B1 activity by a series of estrane-sulfur derivatives (Series 2)

Compound*	**C3-substituent**	**17-O or 17β-OH**	**Inhibition (%) at 0.3 μM****	**Inhibition (%) at 3 μM****
7a	CH_3S	O	13.8 ± 2.5	34.4 ± 2.6
7b	CH_3S	17β-OH	5.1 ± 0.7	26.1 ± 5.0
8a	3-PyrCH_2S	O	**47.7 ± 2.8**	**87.1 ± 0.2**
8b	3-PyrCH_2S	17β-OH	**60.7 ± 2.8**	**94.3 ± 0.6**
9a	CH_3SO	O	15.9 ± 3.3	56.7 ± 0.5
9b	CH_3SO	17β-OH	10.2 ± 1.5	24.5 ± 1.9
10a	CH_3SO_2	O	6.5 ± 4.3	7.2 ± 0.5
10b	CH_3SO_2	17β-OH	0.0 ± 3.3	4.7 ± 3.2
ANF	-	-	94.0 ± 3.2	100.9 ± 0.1

***** See **Scheme 1** for the structure of these compounds.

****** Inhibition of the transformation of resorufin ethyl ether into resorufin by human CYP1B1 in presence of NADPH. The experiment was performed in triplicate (±SD).

2.2. CYP1B1 inhibitory activities of 2-aryl-estrane derivatives

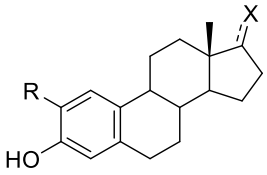
The results obtained with compounds of Series 1 and 2 and the superimposition of the estrane nucleus with the best CYP1B1 inhibitor of the ANF family (**Figure 1C**) oriented our work to the synthesis of a third series of estrane derivatives bearing different phenyl moieties at C2 (compounds **11a-b** to **21a**, **Scheme 2**).

As a first important observation, we can see that almost all compounds of this third series of estrane derivatives are potent CYP1B1 inhibitors with inhibition percentages higher than 68 % at 0.3 μM and a full inhibition of CYP1B1 activity at 3 μM (**Table 2**). Indeed, only compounds **18a**, **19a** and **21a**, with a 4-hydroxyphenyl, 3-methoxyphenyl and 3-aminophenyl moiety, respectively, at C2 of the E1 core, have shown weak inhibitory activities towards CYP1B1 (EROD assay). Another important point is that the ketone **11a**, with a phenyl group at C2 of the steroid core, showed a better CYP1B1 inhibition than those observed with E1-derivatives **1a** and **2a,** bearing respectively a 3- and a 4-pyridinyl at position 2 [27]. Thus, the introduction of a C2-phenyl ring bearing small chemical groups could be a good strategy to optimize the inhibition of estrane derivatives towards CYP1B1. The second major observation is a very interesting gain in CYP1B1 inhibitory activity observed at 0.3 μM for all E2 derivatives (17β-OH), in comparison with their oxidized homologs (E1 derivatives) with a ketone at C17. These results correlate with those previously obtained with Series 1 and 2 and thus confirm the key role that this hydroxy function at C17 plays by promoting the interaction of estrane derivatives with the catalytic site of CYP1B1.

From the results reported in **Table 2**, the best candidates are compounds **13b**, **14b**, **15b**, **16b**, **17a-b** and **20a-b** with CYP1B1 inhibitory activities equal or superior to that of ANF (90.9 ± 1.5 % of inhibition at 0.3 μM). Compound **13b**, with a 4-fluorophenyl core at C2, had the same CYP1B1 inhibitory profile than its analog, compound **14b**, with a 3,4-difluorophenyl moiety (91.1 ± 2.1 and 91.8 ± 1.9 % of inhibition at 0.3 μM, respectively). Compound **12b** bearing a 3-fluorophenyl group was slightly less active than **13b** and **14b**, suggesting that the 4-position on the phenyl group at C2 appears to be the best for the interaction of the fluorine atom with the heme of CYP1B1. Compounds **15b** and **16b**, with a 3- and a 4-chlorophenyl moiety at C2 of the steroid core, respectively, showed close CYP1B1 inhibition potentials (90.5 ± 0.3 and 95.5 ± 2.0 % of inhibition at 0.3 μM, respectively), but the 4-position seems to be slightly advantageous for the chlorine atom. Moreover, compound **16b** with a 4-chlorophenyl group at C2 was more active than its 4-fluoro homolog, **13b**. This

result suggests that the chlorine atom is a better substituent than fluorine for CYP1B1 inhibition.

Table 2. Inhibition of CYP1B1 activity by a series of 2-aryl-estrane derivatives (Series 3)



Compound*	R	X	Inhibition (%) at 0.3 µM**	Inhibition (%) at 3 µM**
1a	3-Pyr	O	37.8 ± 1.5***	89.6 ± 2.2
1b	3-Pyr	17β-OH	85.4 ± 0.3***	100.2 ± 0.1
2a	4-Pyr	O	21.3 ± 3.1***	55.1 ± 11.4
2b	4-Pyr	17β-OH	87.4 ± 0.9***	102.1 ± 0.5
11a	Ph	O	68.5 ± 3.4	100.5 ± 1.5
11b	Ph	17β-OH	82.2 ± 1.9	100.5 ± 0.2
12a	3-F-Ph	O	66.5 ± 6.5	101.3 ± 0.6
12b	3-F-Ph	17β-OH	88.4 ± 0.9	101.0 ± 0.7
13a	4-F-Ph	O	70.8 ± 1.6	100.5 ± 0.2
13b	4-F-Ph	17β-OH	**91.1 ± 2.1**	103.1 ± 0.5
14a	3,4-diF-Ph	O	76.5 ± 1.7	100.4 ± 0.5
14b	3,4-diF-Ph	17β-OH	**91.8 ± 1.9**	100.1 ± 1.0
15a	3-Cl-Ph	O	73.7 ± 0.5	100.2 ± 2.0
15b	3-Cl-Ph	17β-OH	**90.5 ± 0.3**	100.2 ± 1.2
16a	4-Cl-Ph	O	79.2 ± 2.2	100.6 ± 1.8
16b	4-Cl-Ph	17β-OH	**95.5 ± 2.0**	99.1 ± 2.9
17a	3-HO-Ph	O	**91.9 ± 1.8**	103.3 ± 0.3
17b	3-HO-Ph	17β-OH	**96.2 ± 0.7**	100.2 ± 1.4
18a	4-HO-Ph	O	19.3 ± 0.2	30.3 ± 2.0
19a	3-MeO-Ph	O	47.9 ± 4.5	80.8 ± 0.7
20a	4-MeO-Ph	O	**94.8 ± 1.9**	102.8 ± 1.0
20b	4-MeO-Ph	17β-OH	**96.5 ± 1.0**	99.5 ± 1.5
21a	3-H_2N-Ph	O	36.3 ± 4.4	55.2 ± 2.5
ANF	-	-	90.9 ± 1.5	103.8 ± 0.4

***** See **Scheme 2** for the structures of these compounds.

****** Inhibition of the transformation of resorufin ethyl ether into resorufin by human CYP1B1 in presence of NADPH. The experiment was performed in triplicate (±SD).

*** Data from reference 27.

Compounds **17a** and **17b** are the E1- and E2-derivatives with a 3-hydroxyphenyl core at C2 (91.9 ± 1.8 and 96.2 ± 0.7 % of CYP1B1 inhibition at 0.3 μM, respectively). In the same way, **20a** and **20b** are 4-methoxyphenyl derivatives (94.8 ± 1.9 and 96.5 ± 1.0 % of inhibition at 0.3 μM, respectively). If we compare the results obtained for the compounds of Series 3, these four chemicals have shown inhibition percentages superior to that of ANF and close to 100 % when tested at 0.3 μM. Compounds **17b** and **20b** are also the most active CYP1B1 inhibitors of Series 3 but also of the three series of estrane derivatives tested (96.2 ± 0.7 and 96.5 ± 1.0 % of inhibition at 0.3 μM, respectively).

Furthermore, we can see a significant difference in CYP1B1 inhibitory activity for **17a** (3-hydroxyphenyl) and **20a** (4-methoxyphenyl at C2) in comparison with their respective homologs **18a** (4-hydroxyphenyl) and **19a** (3-methoxyphenyl), bearing the same substituent on the phenyl group at C2, but at a distinct position. We observed that the 3-position is more advantageous for the hydroxy function (compounds **17a** *vs* **18a**) while the 4-position is preferable for the methoxy substituent (**19a** *vs* **20a**). Thus, the position of the hydroxy and methoxy functions on the phenyl moiety at C2 appears to have a stronger impact on their activity than the position of halogenated substituents.

CONCLUSION

We synthesized three series of new estrane derivatives in order to assess their inhibitory activity towards CYP1B1: 1) twelve E1 and E2 derivatives bearing 3- and 4-pyridine cores at positions 2, 3 and 4 of the steroid nucleus (Series 1), eight estrane derivatives with different sulfur groups at C3 (Series 2) and nineteen 2-aryl-estrane derivatives (Series 3).

The results obtained with the Series 1 highlighted a very interesting correlation between docking studies and the inhibitory activities measured for this series of pyridine-estrane derivatives, which clearly shows that the position 2 is the best for CYP1B1 inhibition [27]. Compound **1b** (IC_{50} = 0.011 μM), with a 3-pyridine core at C2, was the most active CYP1B1 inhibitor of this series and was more potent than the reference ANF.

Compounds of Series 2 were the less active CYP1B1 inhibitors among the three series of estrane derivatives tested in this study. Only compounds **8a-b**, with a pyridin-3-

ylmethanethio group, showed significant inhibitory activities in this series of 3-thio-estrane derivatives. Thus, the sulfur atom does not seem to be crucial for the interaction of estrane derivatives with CYP1B1. However, the presence of a pyridine core led to a significant increase in inhibitory activity.

The series of 2-aryl-estrane derivatives (Series 3) was based on the superposition of ANF and the steroid core. The results were very promising because almost all the compounds of this third series were potent CYP1B1 inhibitors with an inhibitory activity close to that of ANF. The position 2 of the C18-steroid nucleus thus appears to be the best one to introduce chemical groups such as aryl and pyridine moieties. The most potent CYP1B1 inhibitors of this series and of the three series of estrane derivatives are compounds **17b** and **20b** (96.2 ± 0.7 and 96.5 ± 1.0 % of inhibition at 0.3 μM, respectively) bearing a 3-hydroxyphenyl and a 4-methoxyphenyl group at C2, respectively. As observed for Series 1 and 2, compounds of Series 3 with an hydroxy function at C17 (17β-OH) were more potent CYP1B1 inhibitors than their oxidized homolgs at C17 (ketone at C17) highlighting the key role of 17β-OH for CYP1B1 inhibition, probably due to the formation of a H-bond between 17β-OH and the Asn 228 residue of CYP1B1.

Compounds **17b** and **20b** are thus the most active steroidal CYP1B1 inhibitors to date with an almost full inhibition of CYP1B1 activity at 0.3 μM for both chemicals. It should be emphasized that **20b** is a more attractive candidate than **17b** because it was observed that the adding of methoxy groups to flavonoid derivatives allowed to an increase in selectivity for CYP1B1 *vs* CYP1A1 and CYP1A2 [13, 16]. Four D-ring derivatives of compound **20b**, our best CYP1B1 inhibitor, were also synthesized in order to evaluate the impact of these modifications on the CYP1B1 activity and on the metabolic stability of these estrane derivatives and the results will be reported later.

EXPERIMENTAL SECTION

1. General

Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Dry dichloromethane (DCM) and dimethylformamide (DMF) were obtained from Sigma-Aldrich. Acetonitrile (ACN), ethyl acetate (EtOAc), hexanes and methanol (MeOH)

were obtained from Fisher Scientific (Montréal, Qc, Canada) and were used as received. Reactions using microwave irradiations were performed with a Biotage Initiator (Charlotte, NC, USA). Thin-layer chromatography (TLC) and flash-column chromatography were performed on 0.20 mm silica gel 60 F254 plates (E. Merck; Darmstadt, Germany) and with 230–400 mesh ASTM silica gel 60 (Silicycle, Québec, Qc, Canada), respectively. The purity of final compounds to be tested was determined with a Shimadzu HPLC apparatus (Kyoto, Japan) using a Shimadzu SPD-M20 photodiode array detector, an Alltima HP C18 column (250 mm x 4.6 mm, 5 μm), and a solvent gradient of MeOH:water to MeOH (100%). The wavelength of the UV detector was selected between 190 and 220 nm. Infrared (IR) spectra were recorded on a MB 3000 ABB FTIR spectrometer (Québec, QC, Canada), and only the significant bands are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100.6 MHz for ^{13}C on a Bruker Avance 400 digital spectrometer (Billerica, MA, USA). The chemical shifts (δ) were expressed in ppm and referenced to chloroform (7.26 and 77.0 ppm), dimethylsulfoxide (2.49 and 39.5 ppm) or methanol (3.31 ppm and 49.0 ppm) for ^{1}H and ^{13}C NMR, respectively. Low-resolution mass spectra (LRMS) were recorded on a Shimadzu apparatus (Kyoto, Japan) equipped with an atmospheric pressure chemical ionization (APCI) source. High-resolution mass spectra (HRMS) were provided by Pierre Audet at the Chemistry Department of Université Laval (Québec, Qc, Canada).

2. Synthesis of estrane-sulfur derivatives (Series 2)

*2.1. Synthesis of 3-thiomethyl-estra-1,3,5(10)-triene-17-one (**7a**)*

To a solution of 3-thio-estrone (100 mg, 0.35 mmol) and cesium carbonate (Cs_2CO_3) (228 mg, 0.7 mmol) in ACN (15 mL) was added methyl iodide (MeI) (144 μL, 2.8 mmol). The resulting mixture was stirred and heated under reflux for 2 h. After cooling, the reaction mixture was poured into water and extracted with EtOAc. The organic phase was washed with water, dried over magnesium sulfate ($MgSO_4$), filtered, and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) as eluent to give compound **7a** as a white amorphous solid (70 mg, 67 %). IR (KBr) υ: 1736 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.91 (s, CH_3-18), 1.40-2.45 (m, residual CH and CH_2), 2.47 (s, CH_3S), 2.51 (dd, J_1 = 8.8 Hz, J_2 = 19.0 Hz, 16β-CH), 2.89 (m, CH_2-6), 7.02 (s, CH-4), 7.07 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, CH-2), 7.22 (d, J = 8.2 Hz, CH-1); ^{13}C NMR ($CDCl_3$) δ:

13.8, 16.1, 21.5, 25.7, 26.4, 29.3, 31.5, 35.8, 38.1, 44.1, 47.9, 50.4, 124.5, 125.9, 127.4, 135.3, 136.9, 137.2, 220.8; HRMS for $C_{19}H_{25}OS$ $[M + H]^+$: calc 301.16206, found 301.16237; HPLC purity: 92.4 %.

*2.2. Synthesis of 3-thiomethyl-estra-1,3,5(10)-trien-17β-ol (**7b**)*

To a solution of compound **7a** (105 mg, 0.35 mmol) in MeOH/DCM 9:1 (20 mL) was added under argon atmosphere and at 0 °C sodium borohydride ($NaBH_4$) (12 eq.). The mixture was then stirred at 0 °C under argon for 2 h. The reaction mixture was poured into water and extracted with DCM. The organic phase was washed with water, dried with sodium sulfate (Na_2SO_4) and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) as eluent to give compound **7b** as a white amorphous solid (84 mg, 79 %). IR (KBr) υ: 3379 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.78 (s, CH_3-18), 1.15-2.35 (m, residual CH and CH_2), 2.46 (SCH_3), 2.84 (m, CH_2-6), 3.73 (t, J = 8.4 Hz, 17α-CH), 7.00 (d, J = 1.8 Hz, CH-4), 7.06 (dd, J_1 = 8.2 Hz, J2 = 2.1 Hz, CH-2), 7.22 (d, J = 8.2 Hz, CH-1); ^{13}C NMR ($CDCl_3$) δ: 11.0, 16.2, 23.1, 26.1, 27.1, 29.5, 30.6, 36.7, 38.6, 43.2, 44.1, 50.1, 81.9, 124.5, 125.9, 127.5, 134.9, 137.4, 137.6; HRMS for $C_{19}H_{27}OS$ $[M + H]^+$: calc 303.17771, found 303.17815; HPLC purity: 95.7 %.

*2.3. Synthesis of 3-(pyridin-3-ylmethanethio)-estra-1,3,5(10)-triene-17-one (**8a**)*

To a solution of 3-thioestrone (100 mg, 0.35 mmol), Cs_2CO_3 (456 mg, 1.4 mmol) and tetrabutylammonium iodide (13 mg, 0.035 mmol) in ACN (15 mL) was added 3-(bromomethyl)pyridine hydrobromide (177 mg, 0.7 mmol) and triethylamine (TEA) (0.1 mL, 0.7 mmol). The resulting mixture was then stirred and heated under reflux overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of sodium bicarbonate ($NaHCO_3$) and extracted with EtOAc. The organic phase was washed with water, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM/TEA (99:1) as eluent to give compound **8a** as a light yellow solid (50 mg, 38 %). IR (KBr) υ: 1736 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.91 (s, CH_3-18), 1.35-2.40 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.8 Hz, J_2 = 18.8 Hz, 16β-CH), 2.84 (m, CH_2-6), 4.04 (s, CH_2S), 7.03 (s, CH-4), 7.08 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, CH-2), 7.21 (m, CH-1 and CH of Pyr), 7.63 (dt, J_1 = 7.8 Hz, J_2 = 1.9 Hz, CH of Pyr), 8.43 (d, J = 11.9 Hz, CH of Pyr), 8.47 (dd, J_1 = 4.7 Hz, J_2 = 1.4 Hz, CH of Pyr); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.5, 25.6, 26.3, 29.2, 31.5, 35.8, 36.7, 37.9, 44.2, 47.9, 50.4, 123.5, 126.1, 128.3, 131.5, 131.6, 136.9,

137.5, 139.1, 147.8, 149.3, ~220.0 (very weak); HRMS for $C_{24}H_{28}NOS$ $[M + H]^+$: calc 378.18861, found 378.18819; HPLC purity: 86.7 %.

*2.4. Synthesis of 3-(pyridin-3-ylmethanethio)-estra-1,3,5(10)-trien-17β-ol (**8b**)*

To a solution of compound **8a** (38 mg, 0.1 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (12 eq.). The mixture was then stirred at 0 °C under argon for 3 h. The reaction mixture was poured into water and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with EtOAc/TEA (99:1) as eluent to give compound **8b** as a light yellow solid (20 mg, 52 %). IR (KBr) *v*: 3410 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.78 (s, CH_3-18), 1.15-2.33 (m, residual CH and CH_2), 2.79 (m, CH_2-6), 3.73 (m, 17α-CH), 4.04 (s, CH_2S), 7.02 (s, CH-4), 7.07 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, CH-2), 7.20 (m, CH-1 and CH of Pyr), 7.62 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, CH of Pyr), 8.44 (d, J = 2.0 Hz, CH of Pyr), 8.47 (dd, J_1 = 4.8 Hz, J_2 = 1.5 Hz, CH of Pyr); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.0, 27.0, 29.3, 30.5, 36.6, 36.7, 38.4, 43.2, 44.2, 50.1, 81.8, 123.4, 126.1, 128.2, 131.4, 131.5, 133.9, 136.5, 137.7, 139.7, 148.2, 149.7; HRMS for $C_{24}H_{30}NOS$ $[M + H]^+$: calc 380.20426, found 380.20343; HPLC purity: 93.6 %.

*2.5. Synthesis of 3-methylsulfinyl-estra-1,3,5(10)-triene-17-one (**9a**) and 3-methylsulfonyl-estra-1,3,5(10)-triene-17-one (**10a**)*

To a solution of compound **7a** (50 mg, 0.17 mmol) in MeOH/water 8:2 (10 mL) was added Oxone (104 mg, 0.17 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 2 h, then poured into water and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3), hexanes/EtOAc (5:5) and EtOAc (100 %) as eluent to give compounds **9a** (26 mg, 49 %) and **10a** (15 mg, 27 %) as two white amorphous solids. **9a**: IR (KBr) υ: 1736 (C=O), 1049 (S=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.45-2.48 (m, residual CH and CH_2), 2.52 (dd, J_1 = 8.6 Hz, J_2 = 18.8 Hz, 16β-CH), 2.71 (s, CH_3SO), 3.00 (m, CH_2-6), 7.36 (m, CH-1), 7.43 (m, CH-4 and CH-2); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.5, 25.6, 26.1, 29.4, 31.5, 35.8, 37.8, 43.8, 44.4, 47.8, 50.4, 120.8, 123.8, 126.3, 138.2, 142.6, 143.2, 220.5; HRMS for $C_{19}H_{25}O_2S$ $[M + H]^+$: calc 317.15698, found 317.1561; HPLC purity: 97.1 %. **10a**: IR (KBr) υ: 1744 (C=O), 1142 (S=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.45-2.48 (m, residual CH and CH_2), 2.53 (dd, J_1 = 8.5 Hz, J_2 =

18.8 Hz, 16β-CH), 2.99 (m, CH_2-6), 3.04 (s, CH_3SO_2), 7.48 (d, J = 8.2 Hz, CH-1), 7.67 (s, CH-4), 7.70 (d, J_1 = 8.2 Hz, J_2 = 1.9 Hz, CH-2); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.5, 25.6, 26.0, 29.3, 31.4, 35.7, 37.6, 44.5 (2C), 47.8, 50.4, 124.5, 126.5, 127.7, 137.8, 138.2, 146.2, 220.3; HRMS for $C_{19}H_{25}O_3S$ $[M + H]^+$: calc 333.15189, found 333.15079; HPLC purity: 99.9 %.

*2.6. Synthesis of 3-methylsulfinyl-estra-1,3,5(10)-trien-17β-ol (**9b**) and 3-methylsulfonyl-estra-1,3,5(10)-trien-17β-ol (**10b**)*

To a solution of compound **7b** (80 mg, 0.26 mmol) in MeOH/water 8:2 (16 mL) was added Oxone (160 mg, 0.26 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 2 h, then poured into water and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) to hexanes/EtOAc (5:5) as eluent to give compounds **9b** (22 mg, 26 %) and **10b** (13 mg, 15 %) as two white amorphous solids. **9b**: IR (KBr) υ: 3410 (OH), 1034 (S=O); ^{1}H NMR ($CDCl_3$) δ: 0.79 (s, CH_3-18), 1.17-2.40 (m, residual CH and CH_2), 2.71 (s, CH_3SO), 2.93 (m, CH_2-6), 3.75 (m, 17α-CH), 7.33-7.45 (m, CH-1, CH-2 and CH-4); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.0, 26.8, 29.5, 30.5, 36.6, 38.3, 43.1, 43.8, 44.4, 50.1, 81.7, 120.7, 123.8, 126.4, 138.4, 142.3, 143.8; HRMS for $C_{19}H_{27}O_2S$ $[M + H]^+$: calc 319.17263, found 319.17090; HPLC purity: 93.0 %. **10b**: IR (KBr) υ: 3487 (OH), 1142 (S=O); ^{1}H NMR ($CDCl_3$) δ: 0.79 (s, CH_3-18), 1.20-2.40 (m, residual CH and CH_2), 2.94 (m, CH_2-6), 3.03 (s, CH_3SO_2), 3.75 (t, J = 8.4 Hz, 17α-CH), 7.48 (d, J = 8.2 Hz, CH-1), 7.64 (s, CH-4), 7.68 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, CH-2); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.0, 26.7, 29.4, 30.5, 36.5, 38.1, 43.1, 44.6 (2C), 50.1, 81.7, 124.3, 126.4, 127.6, 137.5, 138.4, 146.9; HRMS for $C_{19}H_{27}O_3S$ $[M + H]^+$: calc 335.16754, found 335.16579; HPLC purity: 95.2 %.

3. Synthesis of 2-aryl-estrane derivatives (Series 3)

*3.1. Synthesis of 2-phenyl-estra-1,3,5(10)-triene-17-one (**11a**)*

To a solution of 2-iodo-3-methoxymethylether(MOM)-estrone(E1) [27] (200 mg, 0.45 mmol) in DMF (3 mL) were added phenylboronic acid (5 eq.), potassium phosphate tribasic (K_3PO_4) (5 eq.) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$) (0.1 eq.). The mixture was then stirred and heated at 120 °C under microwaves

for 4 h. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give 2-phenyl-3-MOM-E1 (100 mg, 56 %). This compound (100 mg, 0.26 mmol) was then dissolved in 15 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with DCM. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) as eluent to give compound **11a** as a white amorphous solid (76 mg, 48 %, 2 steps). IR (KBr) *v*: 3340 (OH), 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.45 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.5 Hz, J_2 = 18.8 Hz, 16β-CH), 2.93 (m, CH_2-6), 5.05 (s, OH), 6.75 (s, CH-4), 7.17 (s, CH-1), 7.38 (m, CH of Ph), 7.46 (m, 4 x CH of Ph); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 26.0, 26.5, 29.3, 31.5, 35.9, 38.4, 43.9, 48.0, 50.4, 115.7, 125.8, 127.2, 127.7, 129.0 (2C), 129.2 (2C), 132.2, 137.3, 137.8, 150.3, 221.0 (very weak); HRMS for $C_{24}H_{27}O_2$ $[M + H]^+$: calc 347.20056, found 347.20123; HPLC purity: 98.5 %.

*3.2. Synthesis of 2-phenyl-estra-1,3,5(10)-trien-17β-ol (**11b**)*

To a solution of compound **11a** (65 mg, 0.19 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (12 eq.). The mixture was stirred at 0 °C under argon for 2 h, then poured into water and extracted with DCM. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) as eluent to give compound **11b** as a yellow amorphous solid (63 mg, 96 %). IR (KBr) *v*: 3387 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.79 (s, CH_3-18), 1.15-2.35 (m, residual CH and CH_2), 2.88 (m, CH_2-6), 3.73 (t, J = 8.4 Hz, 17α-CH), 5.04 (s, 3-OH), 6.73 (s, CH-4), 7.17 (s, CH-1), 7.38 (m, CH of Ph), 7.47 (m, 4 x CH of Ph); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.3, 27.2, 29.4, 30.5, 36.6, 38.8, 43.2, 43.9, 50.0, 81.9, 115.7, 125.6, 127.2, 127.5 129.1 (4C), 132.8, 137.5, 138.0, 150.2; HRMS for $C_{24}H_{29}O_2$ $[M + H]^+$: calc 349.21621, found 349.21683; HPLC purity: 99.2 %.

*3.3. Synthesis of 2-(3-fluorophenyl)-estra-1,3,5(10)-triene-17-one (**12a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 3-fluorophenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and $Pd(dppf)Cl_2$ (0.1 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 4 h. After cooling, the reaction mixture was poured into water and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1 to 7:3) as eluent to give 2-(3-fluorophenyl)-3-MOM-E1 (160 mg, 86 %). This compound (160 mg, 0.39 mmol) was then dissolved in 20 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) as eluent to give compound **12a** as a white-orange amorphous solid (100 mg, 60 %, 2 steps). IR (KBr) *v*: 3356 (OH), 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.48 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.6 Hz, J_2 = 18.7 Hz, 16β-CH), 2.92 (m, CH_2-6), 4.96 (s, OH), 6.73 (s, CH-4), 7.07 (td, J_1 = 8.4 Hz, J_2 = 2.2 Hz, CH of Ar), 7.16 (s, CH-1), 7.19 (m, CH of Ar), 7.24 (m, CH of Ar), 7.43 (td, J_1 = 8.0 Hz, J_2 = 6.1 Hz, CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 26.0, 26.5, 29.2, 31.5, 35.9, 38.3, 43.9, 48.0, 50.4, 114.4 (d, J_{CCF} = 21.1 Hz), 116.0, 116.1, 124.6, 127.2, 130.5 (d, J_{CCCF} = 8.4 Hz), 132.5, 138.2, 139.7 (d, J_{CCCF} = 8.0 Hz), 150.2, 163.1 (d, J_{CF} = 247.3 Hz), 221.0; HRMS for $C_{24}H_{26}FO_2$ $[M + H]^+$: calc 365.19113, found 365.19192; HPLC purity: 92.5 %.

*3.4. Synthesis of 2-(3-fluorophenyl)-estra-1,3,5(10)-trien-17β-ol (**12b**)*

To a solution of compound **12a** (40 mg, 0.11 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (10 eq.). The mixture was stirred at 0 °C under argon for 2 h, then poured into water and extracted with DCM. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) as eluent to give compound **12b** as a white-yellow amorphous solid (30 mg, 75 %). IR (KBr) *v*: 3394 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.79 (s, CH_3-18), 1.15-2.35 (m, residual CH and CH_2), 2.87 (m, CH_2-6), 3.74 (t, J = 8.4 Hz, 17α-CH), 6.70 (s, CH-4), 7.06 (m, CH of Ar), 7.16 (s, CH-1), 7.17-7.27 (m, 2 x CH of Ar), 7.42 (td, J_1 = 8.0 Hz, J_2 = 6.0 Hz, CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.4, 27.1, 29.4, 30.6, 36.6, 38.8, 43.2, 43.9, 50.0, 81.9, 114.3 (d, J_{CCF} = 21.0 Hz), 115.9,

116.2 (d, J_{CCF} = 21.3 Hz), 124.4, 124.6, 127.2, 130.5 (d, J_{CCCF} = 8.4 Hz), 133.1, 138.5, 139.9 (d, J_{CCCF} = 7.5 Hz), 150.0, 161.9 (d, J_{CF} = 247.1 Hz); HRMS for $C_{24}H_{28}FO_2$ $[M + H]^+$: calc 367.20678, found 367.20734; HPLC purity: 93.0 %.

*3.5. Synthesis of 2-(4-fluorophenyl)-estra-1,3,5(10)-triene-17-one (**13a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 4-fluorophenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and Pd(dppf)Cl_2 (0.1 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 4 h. After cooling, the reaction mixture was poured into water and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (95:5 to 9:1) as eluent to give 2-(4-fluorophenyl)-3-MOM-E1 (105 mg, 57 %). This compound (105 mg, 0.26 mmol) was then dissolved in 15 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) as eluent and a second time with DCM/MeOH (99:1 to 95:5) as eluent to give compound **13a** as a white amorphous solid (62 mg, 37 %, 2 steps). IR (KBr) *v*: 3364 (OH), 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.48 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.5 Hz, J_2 = 18.7 Hz, 16β-CH), 2.92 (m, CH_2-6), 4.90 (s, OH), 6.72 (s, CH-4), 7.13 (s, CH-1), 7.15 (m, 2 x CH of Ar), 7.43 (m, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 26.0, 26.5, 29.2, 31.5, 35.9, 38.3, 43.9, 48.0, 50.4, 115.8, 116.0 (d, J_{CCF} = 21.5 Hz, 2C), 124.9, 127.3, 130.8 (d, J_{CCCF} = 7.8 Hz, 2C), 132.4, 133.4, 137.8, 150.3, 162.3 (d, J_{CF} = 246.7 Hz), 221.9 (very weak); HRMS for $C_{24}H_{26}FO_2$ $[M + H]^+$: calc 365.19113, found 365.19218; HPLC purity: 99.8 %.

*3.6. Synthesis of 2-(4-fluorophenyl)-estra-1,3,5(10)-trien-17β-ol (**13b**)*

To a solution of compound **13a** (33 mg, 0.09 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (10 eq.). The mixture was stirred at 0 °C under argon for 2 h, then poured into water and extracted with DCM. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) as eluent to give compound **13b** as a yellow amorphous solid (28 mg, 80 %). IR (KBr) *v*: 3394 (OH); ^{1}H NMR

($CDCl_3$) δ: 0.79 (s, CH_3-18), 1.15-2.35 (m, residual CH and CH_2), 2.87 (m, CH_2-6), 3.74 (t, J = 8.4 Hz, 17α-CH), 4.88 (s, 3-OH), 6.70 (s, CH-4), 7.15 (m, CH-1 and 2 x CH of Ar), 7.43 (m, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.4, 27.2, 29.3, 30.5, 36.6, 38.8, 43.2, 43.9, 50.0, 81.9, 115.8, 115.9 (d, J_{CCF} = 20.5 Hz, 2C), 124.7, 127.3, 130.8 (d, J_{CCCF} = 8.2 Hz, 2C), 132.9, 133.5, 138.0, 150.1, 162.2 (d, J_{CF} = 246.6 Hz); HRMS for $C_{24}H_{28}FO_2$ $[M + H]^+$: calc 367.20678, found 367.20719; HPLC purity: 98.2 %.

*3.7. Synthesis of 2-(3,4-difluorophenyl)-estra-1,3,5(10)-triene-17-one (**14a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 3,4-difluorophenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and Pd(dppf)Cl_2 (0.05 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 4 h. After cooling, the reaction mixture was quenched with a saturated aqueous solution of NaCl and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give 2-(3,4-difluorophenyl)-3-MOM-E1 (105 mg, 54 %). This compound (105 mg, 0.25 mmol) was then dissolved in 15 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM (100 %) as eluent to give compound **14a** as a white amorphous solid (85 mg, 49 %, 2 steps). IR (KBr) *ν*: 3425 (OH), 1720 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.48 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.6 Hz, J_2 = 18.8 Hz, 16β-CH), 2.91 (m, CH_2-6), 4.88 (s, OH), 6.70 (s, CH-4), 7.13 (s, CH-1), 7.19-7.34 (m, 3 x CH of Ar); ^{13}C NMR (MeOD/$CDCl_3$ 1:1) δ: 14.2, 22.2, 26.6, 27.2, 29.8, 32.2, 36.5, 39.2, 44.6, 48.9, 51.1, 116.6, 117.2 (d, J_{CCF} = 17.1 Hz), 118.8 (d, J_{CCF} = 17.5 Hz), 124.8, 125.9 (dd, J_{CCCF} = 6.1 Hz, J_{CCCCF} = 3.4 Hz), 127.9, 132.0, 137.0 (dd, J_{CCCF} = 6.3 Hz, J_{CCCCF} = 3.8 Hz), 138.3, 149.8 (dd, J_{CF} = 246.2 Hz, J_{CCF} = 12.6 Hz), 150.5 (dd, J_{CF} = 245.5 Hz, J_{CCF} = 12.7 Hz), 152.5, 223.6; HRMS for $C_{24}H_{25}F_2O_2$ $[M + H]^+$: calc 383.18171, found 383.18191; HPLC purity: 99.1 %.

*3.8. Synthesis of 2-(3,4-difluorophenyl)-estra-1,3,5(10)-trien-17β-ol (**14b**)*

To a solution of compound **14a** (40 mg, 0.10 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (10 eq.). The mixture was stirred at 0 °C

under argon for 2 h, then poured into water and extracted with DCM. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) as eluent to give compound **14b** as a white-yellow amorphous solid (37 mg, 92 %). IR (KBr) *v*: 3387 (OH); ^{1}H NMR ($CD_3OD/CDCl_3$ 1:1) δ: 0.75 (s, CH_3-18), 1.12-2.35 (m, residual CH and CH_2), 2.81 (m, CH_2-6), 3.65 (t, J = 8.6 Hz, 17α-CH), 6.61 (s, CH-4), 7.12 (s, CH-1), 7.10-7.42 (m, 3 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 11.4, 23.5, 27.0, 27.8, 29.9, 30.2, 37.2, 39.6, 43.7, 44.5, 50.6, 81.8, 116.5, 117.0 (d, J_{CCF} = 17.1 Hz), 118.7 (d, J_{CCF} = 17.5 Hz), 124.5, 125.8 (dd, J_{CCCF} = 6.1 Hz, J_{CCCCF} = 3.3 Hz), 127.9, 132.6, 136.9 (dd, J_{CCCF} = 6.3 Hz, J_{CCCCF} = 4.2 Hz), 138.4, 149.7 (dd, J_{CF} = 246.3 Hz, J_{CCF} = 12.7 Hz), 150.3 (dd, J_{CF} = 245.6 Hz, J_{CCF} = 12.7), 152.1; HRMS for $C_{24}H_{27}F_2O_2$ $[M + H]^+$: calc 385.19736, found 385.19770; HPLC purity: 97.9 %.

*3.9. Synthesis of 2-(3-chlorophenyl)-estra-1,3,5(10)-triene-17-one (**15a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 3-chlorophenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and $Pd(dppf)Cl_2$ (0.1 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 4 h. After cooling, the reaction mixture was poured into water and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give 2-(3-chlorophenyl)-3-MOM-E1 (105 mg, 54 %). This compound (105 mg, 0.25 mmol) was then dissolved in 15 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM (100 %) to DCM-MeOH (97:3) as eluent to give compound **15a** as a light yellow amorphous solid (73 mg, 42 %, 2 steps). IR (KBr) *v*: 3356 (OH), 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.48 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.6 Hz, J_2 = 18.8 Hz, 16β-CH), 2.92 (m, CH_2-6), 5.04 (s, OH), 6.72 (s, CH-4), 7.15 (s, CH-1), 7.36 (m, 3 x CH of Ar), 7.46 (s, CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 26.0, 26.4, 29.2, 31.5, 35.9, 38.3, 43.9, 48.0, 50.4, 116.0, 124.5, 127.1, 127.2, 127.6, 129.2, 130.1, 132.5, 134.8, 138.2, 139.4, 150.2, 221.1; HRMS for $C_{24}H_{26}ClO_2$ $[M + H]^+$: calc 381.16158, found 381.16230; HPLC purity: 94.6 %.

*3.10. Synthesis of 2-(3-chlorophenyl)-estra-1,3,5(10)-trien-17β-ol (**15b**)*

To a solution of compound **15a** (24 mg, 0.06 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (10 eq.). The mixture was stirred at 0 °C under argon for 2 h, then poured into water and extracted with DCM. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) as eluent to give compound **15b** as a light yellow amorphous solid (20 mg, 83 %). IR (KBr) *v*: 3256 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.79 (s, CH_3-18), 1.15-2.35 (m, residual CH and CH_2), 2.87 (m, CH_2-6), 3.74 (t, J = 8.4 Hz, 17α-CH), 6.70 (s, CH-4), 7.15 (s, CH-1), 7.35 (m, 3 x CH of Ar), 7.47 (s, CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.4, 27.1, 29.3, 30.5, 36.6, 38.8, 43.2, 43.9, 50.0, 81.9, 115.9, 124.3, 127.2 (2C), 127.5, 129.3, 130.1, 133.1, 134.8, 138.5, 139.6, 150.0; HRMS for $C_{24}H_{28}ClO_2$ $[M + H]^+$: calc 383.17723, found 383.17698; HPLC purity: 89.5 %.

*3.11. Synthesis of 2-(4-chlorophenyl)-estra-1,3,5(10)-triene-17-one (**16a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 4-chlorophenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and $Pd(dppf)Cl_2$ (0.05 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 3 h. After cooling, the reaction mixture was poured into water and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give 2-(4-chlorophenyl)-3-MOM-E1 (105 mg, 65 %). This compound (126 mg, 0.30 mmol) was then dissolved in 15 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM (100 %) as eluent to give compound **16a** as a white amorphous solid (87 mg, 50 %, 2 steps). IR (KBr) *v*: 3425 (OH), 1720 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.45 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.5 Hz, J_2 = 18.7 Hz, 16β-CH), 2.92 (m, CH_2-6), 4.90 (s, OH), 6.72 (s, CH-4), 7.14 (s, CH-1), 7.38-7.46 (m, 4 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 26.0, 26.5, 29.2, 31.5, 35.9, 38.3, 43.9, 48.0, 50.4, 115.9, 124.7, 127.3, 129.1 (2C), 130.4 (2C), 132.5, 133.5, 135.9, 138.0, 150.2, 221.1; HRMS for $C_{24}H_{26}ClO_2$ $[M + H]^+$: calc 381.16158, found 381.16255; HPLC purity: 96.7 %.

*3.12. Synthesis of 2-(4-chlorophenyl)-estra-1,3,5(10)-trien-17β-ol (**16b**)*

To a solution of compound **16a** (40 mg, 0.11 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (10 eq.). The mixture was stirred at 0 °C under argon for 2 h, then poured into water and extracted with DCM. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) as eluent to give compound **16b** as a yellow-orange amorphous solid (34 mg, 85 %). IR (KBr) *v*: 3410 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.79 (s, CH_3-18), 1.15-2.35 (m, residual CH and CH_2), 2.87 (m, CH_2-6), 3.74 (t, J = 8.5 Hz, 17α-CH), 6.69 (s, CH-4), 7.14 (s, CH-1), 7.42 (s, 4 x CH of Ar); ^{13}C NMR ($CD_3OD/CDCl_3$ 1:1) δ: 11.5, 23.6, 27.0, 27.9, 29.9, 30.2, 37.3, 39.7, 43.8, 44.6, 50.7, 81.9, 116.5, 125.5, 128.0, 128.6 (2C), 131.2 (2C), 132.7, 132.8, 138.2, 138.4, 152.2; HRMS for $C_{24}H_{28}ClO_2$ $[M + H]^+$: calc 383.17723, found 383.17634; HPLC purity: 90.9 %.

*3.13. Synthesis of 2-(3-hydroxyphenyl)-estra-1,3,5(10)-triene-17-one (**17a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 3-hydroxyphenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and Pd(dppf)Cl_2 (0.05 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 4 h. After cooling, the reaction mixture was poured into water, neutralized with a solution of 10 % aqueous HCl and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2 to 7:3) as eluent to give 2-(3-hydroxyphenyl)-3-MOM-E1 (130 mg, 70 %). This compound (130 mg, 0.32 mmol) was then dissolved in 15 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was poured into water, neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM (100 %) to DCM-MeOH (98:2) as eluent to give compound **17a** as a white-yellow amorphous solid (94 mg, 57 %, 2 steps). IR (KBr) *v*: 3448 (OH), 1720 (C=O); ^{1}H NMR (CD_3OD) δ: 0.93 (s, CH_3-18), 1.35-2.45 (m, residual CH and CH_2), 2.49 (dd, J_1 = 8.5 Hz, J_2 = 18.3 Hz, 16β-CH), 2.86 (m, CH_2-6), 6.59 (s, CH-4), 6.69 (m, CH of Ar), 6.97 (m, 2 x CH of Ar), 7.12 (s, CH-1), 7.17 (t, J = 7.8 Hz, CH of Ar); ^{13}C NMR ($CD_3OD/CDCl_3$ 1:1) δ: 14.2, 22.1, 26.5, 27.1, 29.7, 32.1, 36.4, 39.1, 44.5, 51.0, 50.4, 114.2, 116.4, 116.8, 121.3, 126.9, 128.1, 129.7, 131.7, 137.5,

140.9, 152.1, 157.1, 223.7; HRMS for $C_{24}H_{27}O_3$ $[M + H]^+$: calc 363.19547, found 363.19619; HPLC purity: 99.3 %.

*3.14. Synthesis of 2-(3-hydroxyphenyl)-estra-1,3,5(10)-trien-17β-ol (**17b**)*

To a solution of compound **17a** (40 mg, 0.11 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (10 eq.). The mixture was stirred at 0 °C under argon for 3 h, then poured into water, neutralized with a solution of 10 % aqueous HCl and extracted 3 times with DCM and 3 times with EtOAc. Each organic phase was washed with water, the 2 organic phases were then combined, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) as eluent to give compound **17b** as a yellow-orange amorphous solid (21 mg, 52 %). IR (KBr) *v*: 3394 (OH); ^{1}H NMR ($CD_3OD/CDCl_3$) δ: 0.75 (s, CH_3-18), 1.15-2.35 (m, residual CH and CH_2), 2.80 (m, CH_2-6), 3.65 (t, J = 8.5 Hz, 17α-CH), 6.60 (s, CH-4), 6.75 (m, CH of Ar), 7.00 (m, 2 x CH of Ar), 7.14 (s, CH-1), 7.19 (t, J = 7.8 Hz, CH of Ar); ^{13}C NMR ($CD_3OD/CDCl_3$ 1:1) δ: 11.4, 23.5, 26.9, 27.9, 29.9, 30.2, 37.2, 39.6, 43.7, 44.6, 50.6, 81.9, 114.1, 116.3, 116.8, 121.3, 126.7, 128.1, 129.6, 132.5, 137.7, 141.0, 151.8, 157.1; HRMS for $C_{24}H_{29}O_3$ $[M + H]^+$: calc 365.21112, found 365.21199; HPLC purity: 97.1 %.

*3.15. Synthesis of 2-(4-hydroxyphenyl)-estra-1,3,5(10)-triene-17-one (**18a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 4-hydroxyphenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and $Pd(dppf)Cl_2$ (0.05 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 3 h. After cooling, the reaction mixture was poured into water, neutralized with a solution of 10 % aqueous HCl and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2 to 6:4) as eluent to give 2-(4-hydroxyphenyl)-3-MOM-E1 (150 mg, 81 %). This compound (150 mg, 0.37 mmol) was then dissolved in 15 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was poured into water, neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM (100 %), DCM/MeOH (99.5:0.5) and DCM/MeOH (99:1) as eluent to give compound **18a** as a light

brown amorphous solid (110 mg, 67 %, 2 steps). IR (KBr) *v*: 3317 (OH), 1720 (C=O); ^{1}H NMR (CD_3OD) δ: 0.93 (s, CH_3-18), 1.35-2.45 (m, residual CH and CH_2), 2.49 (dd, J_1 = 8.5 Hz, J_2 = 18.4 Hz, 16β-CH), 2.85 (m, CH_2-6), 6.57 (s, CH-4), 6.79 (d, J = 8.7 Hz, 2 x CH of Ar), 7.09 (s, CH-1), 7.34 (d, J = 8.7 Hz, 2 x CH of Ar); ^{13}C NMR ($CD_3OD/CDCl_3$ 1:1) δ: 14.2, 22.1, 26.5, 27.2, 29.7, 32.1, 36.5, 39.1, 44.6, 48.8, 51.0, 115.5 (2C), 116.3, 126.9, 128.0, 130.8, 130.9 (2C), 131.7, 136.8, 152.1, 156.3, 223.7; HRMS for $C_{24}H_{27}O_3$ $[M + H]^+$: calc 363.19547, found 363.19603; HPLC purity: 98.7 %.

*3.16. Synthesis of 2-(3-methoxyphenyl)-estra-1,3,5(10)-triene-17-one (**19a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 3-methoxyphenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and Pd(dppf)Cl_2 (0.05 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 4 h. After cooling, the reaction mixture was poured into water and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give 2-(3-methoxyphenyl)-3-MOM-E1 (140 mg, 73 %). This compound (140 mg, 0.33 mmol) was then dissolved in 20 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM (100 %) to DCM-MeOH (99:1) as eluent to give compound **19a** as a white solid (89 mg, 52 %, 2 steps). IR (KBr) *v*: 3325 (OH), 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.45 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.6 Hz, J_2 = 18.7 Hz, 16β-CH), 2.93 (m, CH_2-6), 3.84 (s, CH_3O), 5.21 (s, OH), 6.75 (s, CH-4), 6.93 (dd, J_1 = 8.2 Hz, J_2 = 2.4 Hz, CH of Ar), 6.97 (t, J = 2.3 Hz, CH of Ar), 7.03 (d, J = 7.6 Hz, CH of Ar, 7.17 (s, CH-1), 7.39 (t, J = 7.9 Hz, CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 25.9, 26.5, 29.2, 31.5, 35.8, 38.3, 43.9, 48.0, 50.4, 55.3, 113.2, 114.6, 115.7, 121.1, 125.6, 127.0, 130.3, 132.1, 137.8, 138.7, 150.3, 160.2, 221.1; HRMS for $C_{25}H_{29}O_3$ $[M + H]^+$: calc 377.21112, found 377.21250; HPLC purity: 99.8 %.

*3.17. Synthesis of 2-(4-methoxyphenyl)-estra-1,3,5(10)-triene-17-one (**20a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 4-methoxyphenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and Pd(dppf)Cl_2 (0.05 eq.). The mixture

was then stirred and heated at 120 °C under microwaves for 3.5 h. After cooling, the reaction mixture was poured into water and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give 2-(4-methoxyphenyl)-3-MOM-E1 (174 mg, 91 %). This compound (174 mg, 0.41 mmol) was then dissolved in 20 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM (100 %) to DCM/MeOH (99:1) as eluent to give compound **20a** as a light yellow amorphous solid (122 mg, 71 %, 2 steps). IR (KBr) *v*: 3379 (OH), 1720 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.45 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.6 Hz, J_2 = 18.7 Hz, 16β-CH), 2.92 (m, CH_2-6), 3.86 (s, CH_3O), 5.04 (s, OH), 6.73 (s, CH-4), 7.01 (d, J = 8.8 Hz, 2 x CH of Ar), 7.13 (s, CH-1), 7.37 (d, J = 8.7 Hz, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 13.8, 21.6, 26.0, 26.5, 29.2, 31.5, 35.9, 38.4, 43.9, 48.0, 50.4, 55.4, 114.6 (2C), 115.5, 125.5, 127.2, 129.4, 130.2 (2C), 132.1, 137.3, 150.4, 159.2, 221.1 (very weak); HRMS for $C_{25}H_{29}O_3$ $[M + H]^+$: calc 377.21112, found 377.21202; HPLC purity: 99.7 %.

*3.18. Synthesis of 2-(4-methoxyphenyl)-estra-1,3,5(10)-trien-17β-ol (**20b**)*

To a solution of compound **20a** (50 mg, 0.13 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (10 eq.). The mixture was stirred at 0 °C under argon for 2.5 h, then poured into water and extracted with DCM. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) as eluent to give compound **20b** as an orange amorphous solid (45 mg, 90 %). IR (KBr) *v*: 3371 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.79 (s, CH_3-18), 1.15-2.35 (m, residual CH and CH_2), 2.87 (m, CH_2-6), 3.73 (t, J = 8.5 Hz, 17α-CH), 3.85 (s CH_3O), 6.71 (s, CH-4), 7.00 (d, J = 8.7 Hz, 2 x CH of Ar), 7.14 (s, CH-1), 7.38 (d, J = 8.7 Hz, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.4, 27.2, 29.4, 30.6, 36.7, 38.8, 43.2, 43.9, 50.0, 55.4, 81.9, 114.6 (2C), 115.5, 125.3, 127.2, 129.6, 130.2 (2C), 132.7, 137.6, 150.2, 159.1; HRMS for $C_{25}H_{31}O_3$ $[M + H]^+$: calc 379.22677, found 379.22766; HPLC purity: 97.6 %.

*3.19. Synthesis of 2-(3-aminophenyl)-estra-1,3,5(10)-triene-17-one (**21a**)*

To a solution of 2-I-3-MOM-E1 (200 mg, 0.45 mmol) in DMF (3 mL) were added 3-aminophenylboronic acid (5 eq.), K_3PO_4 (5 eq.) and $Pd(dppf)Cl_2$ (0.05 eq.). The mixture was then stirred and heated at 120 °C under microwaves for 3 h. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (7:3) + 1 % of TEA as eluent to give 2-(3-aminophenyl)-3-MOM-E1 (70 mg, 38 %). This compound (70 mg, 0.17 mmol) was then dissolved in 10 mL of a solution of 10 % aqueous HCl in MeOH (1:9). The resulting mixture was stirred and heated at 50 °C overnight. After cooling, the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was washed with water, dried with $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with DCM/TEA (99:1) as eluent to give compound **21a** as a white-yellow amorphous solid (42 mg, 26 %, 2 steps). IR (KBr) *v*: 3518 and 3464 (NH_2), 3371 OH), 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.45 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.5 Hz, J_2 = 18.7 Hz, 16β-CH), 2.92 (m, CH_2-6), 3.78 (s, NH_2), 5.27 (s, OH), 6.71 (m, 2 x CH of Ar), 6.74 (s, CH-4), 6.81 (d, J = 7.5 Hz, CH of Ar), 7.15 (s, CH-1), 7.25 (t, J = 7.8 Hz, CH of Ar); ^{13}C NMR (DMSO-d_6) δ: 13.5, 21.2, 25.7, 26.2, 28.7, 31.4, 35.4, 38.0, 43.4, 47.4, 49.6, 112.0, 114.9, 115.7, 117.0, 126.1, 126.9, 128.2, 130.2, 135.9, 139.6, 148.1, 152.0, 219.8; HRMS for $C_{24}H_{28}NO_2$ $[M + H]^+$: calc 362.21146, found 362.21246; HPLC purity: 94.6 %.

4. Synthesis of D-ring derivatives of compound 20b (compounds 23, 24, 25 and 26)

*4.1. Synthesis of 2-(4-methoxyphenyl)-3-methoxy-estra-1,3,5(10)-triene-17-one (**22**)*

To a solution of compound **20a** (250 mg, 0.66 mmol) and Cs_2CO_3 (2 eq.) in ACN (25 mL) was added methyl iodide (10 eq.). The mixture was then stirred and heated to reflux for 3 h. After cooling, the reaction mixture was quenched with water and extracted with EtOAc. The organic phase was washed with water, dried over $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1 to 8:2) and then with DCM-MeOH (95:5) as eluent to give compound **22** as a white amorphous solid (223 mg, 86 %). IR (KBr) v: 1728 (C=O); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, CH_3-18), 1.40-2.45 (m, residual CH and CH_2), 2.51 (dd, J_1 = 8.6 Hz and J_2 = 18.7 Hz, CH-16β),

2.97 (m, CH_2-6), 3.78 (s, 3-CH_3O), 3.84 (s, CH_3O of Ar), 6.71 (s, CH-4), 6.94 (d, J = 8.8 Hz, 2 x CH of Ar) 7.22 (s, CH-1), 7.44 (d, J = 8.7 Hz, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 13.9, 21.6, 26.0, 26.6, 29.6, 31.6, 35.9, 38.4, 44.0, 48.0, 50.4, 55.3, 55.6, 111.5, 113.5 (2C), 127.8, 128.0, 130.5 (2C), 131.0, 131.9, 136.4, 154.4, 158.5, 220.9 (very weak); LRMS for $C_{26}H_{31}O_3$ $[M + H]^+$: 441.0.

*4.2. Synthesis of 2-(4-methoxyphenyl)-3-methoxy-17α-methyl-estra-1,3,5(10)-trien-17β-ol (**23**)*

To a solution of compound **22** (80 mg, 0.20 mmol) in anhydrous toluene (3 mL) was added under argon atmosphere 0.7 mL of a solution of methylmagnesium iodide (3.0 M in diethyl ether) (10 eq.). The resulting mixture was stirred and heated at 80 °C for 4 h and then at room temperature overnight. The reaction mixture was quenched with a saturated solution of NH_4Cl, extracted 3 times with DCM and 2 times with EtOAc. Each organic phase was washed with water, combined together, dried over $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) as eluent to give compound **23** as a white amorphous solid (65 mg, 78 %). IR (KBr) *v*: 3433 (OH); ^1H NMR ($CDCl_3$) δ: 0.90 (s, CH_3-18), 1.28 (s, 17α-CH_3), 1.30-2.40 (m, residual CH and CH_2), 2.92 (m, CH_2-6), 3.78 (s, 3-CH_3O), 3.84 (s, CH_3O of Ar), 6.69 (s, CH-4), 6.94 (d, J = 8.8 Hz, 2 x CH of Ar), 7.22 (s, CH-1), 7.45 (d, J = 8.8 Hz, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 13.9, 22.9, 25.8, 26.3, 27.5, 29.8, 31.7, 39.0, 39.7, 43.8, 45.8, 49.6, 55.3, 55.6, 81.7, 111.5, 113.4 (2C), 127.7, 127.8, 130.5 (2C), 131.2, 132.5, 136.7, 154.3, 158.4; HRMS for $C_{27}H_{35}O_3$ $[M + H]^+$: calc 407.25806, found 407.25948; HPLC purity: 96.9 %.

*4.3. Synthesis of 2-(4-methoxyphenyl)-3-methoxy-17α-ethynyl-estra-1,3,5(10)-trien-17β-ol (**24**)*

To a solution of trimethylsilylacetylene (0.1 mL, 0.80 mmol) (4 eq.) in anhydrous diethyl ether (6 mL) under argon atmosphere was added MeLi (0.4 mL, 0.60 mmol, from 1.6 M solution in diethyl ether) (3 eq.) at 0 ºC. The solution was stirred at room temperature for 1 h and cooled again at 0 ºC before the addition of a solution of compound **22** (80 mg, 0.2 mmol) in anhydrous THF (6 mL). The resulting solution was allowed to return to room temperature and stirred overnight. The solution was then poured into water, extracted with EtOAc, washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude compound was dissolved in a 5 % K_2CO_3 solution in MeOH (10 mL) and

stirred overnight. The reaction mixture was then poured into water, neutralised to pH 7 with an aqueous solution of HCl 10 %, extracted 3 times with EtOAc and 2 times with DCM. Each organic phase was washed with water, combined together, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1) as eluent to give the desired compound **24** as an amorphous light yellow solid (27 mg, 32 %). IR (KBr) *v*: 3441 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.35-2.45 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.92 (m, CH_2-6), 3.78 (s, 3-CH_3O), 3.84 (s, CH_3O of Ar), 6.69 (s, CH-4), 6.94 (d, J = 8.8 Hz, 2 x CH of Ar), 7.23 (s, CH-1), 7.45 (d, J = 8.8 Hz, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 12.7, 22.8, 26.4, 27.3, 29.8, 32.7, 38.9, 39.5, 43.5, 47.1, 49.4, 55.3, 55.6, 74.1, 79.9, 87.5, 111.5, 113.4 (2C), 127.8, 127.9, 130.5 (2C), 131.2, 132.4, 136.7, 154.3, 158.5; HPLC purity: 97.7 %.

*4.4. Synthesis of 2-(4-methoxyphenyl)-17α-ethynyl-estra-1,3,5(10)-trien-17β-ol (**25**)*

To a solution of trimethylsilylacetylene (0.06 mL, 0.44 mmol) (4 eq.) in anhydrous diethyl ether (3 mL) under an argon atmosphere was added MeLi (0.2 mL, 0.33 mmol, from 1.6 M solution in diethy ether) (3 eq.) at 0 ºC. The solution was stirred at room temperature for 1 h and cooled again at 0 ºC before the addition of a solution of compound **20a** (40 mg, 0.11 mmol) in anhydrous THF (3 mL). The resulting solution was allowed to return to room temperature and stirred overnight. The solution was then poured into water, extracted with EtOAc, washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude compound was dissolved in a 5 % K_2CO_3 solution in MeOH (5 mL) and stirred for 5 h. The reaction mixture was then poured into water, neutralised to pH 7 with an aqueous solution of HCl 10 %, extracted with EtOAc. The organic phase was washed with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (9:1 to 8:2) as eluent to give the desired compound **25** as a yellow-orange amorphous solid (21 mg, 49 %). IR (KBr) *v*: 3410 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.89 (s, CH_3-18), 1.30-2.40 (m, residual CH and CH_2), 2.61 (s, C≡CH), 2.87 (m, CH_2-6), 3.36 (s, 17β-OH), 3.86 (s, CH_3O of Ar), 5.00 (s, 3-OH), 6.71 (s, CH-4), 7.01 (d, J = 8.7 Hz, 2 x CH of Ar), 7.14 (s, CH-1), 7.38 (d, J = 8.7 Hz, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 12.7, 22.8, 26.4, 27.2, 29.4, 32.7, 38.9, 39.4, 43.5, 47.1, 49.4, 55.4, 74.1, 79.9, 87.5, 114.6 (2C), 115.5, 125.3, 127.2, 129.6, 130.2 (2C), 132.6, 137.6, 150.2, 159.1; HRMS for $C_{27}H_{31}O_3$ $[M + H]^+$: calc 403.22677, found 403.22667; HPLC purity: 95.6 %.

*4.5. Synthesis of 2-(4-methoxyphenyl)-3,17β-dimethoxy-estra-1,3,5(10)-trien-17β-ol (**26**)*

To a solution of compound **20b** (30 mg, 0.08 mmol) in anhydrous DMF (5 mL) were added under argon atmosphere NaH (60 % in oil, 30 mg, 7 eq.). The resulting mixture was stirred for 1 h at 0 °C before the addition of methyl iodide (0.1 mL, 20 eq.). The mixture was then stirred at room temperature overnight. The reaction mixture was poured into a saturated aqueous solution of NH_4Cl and extracted with EtOAc. The organic phase was washed with water, dried over $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography with hexanes/EtOAc (8:2) as eluent to give compound **26** as an amorphous brown solid (15 mg, 47 %). IR (KBr) *v*: 3387 (OH); ^{1}H NMR ($CDCl_3$) δ: 0.80 (s, CH_3-18), 1.20-2.35 (m, residual CH and CH_2), 2.90 (m, CH_2-6), 3.32 (t, J = 8.3 Hz, 17α-CH), 3.38 (s, 17-CH_3O), 3.78 (s, 3-CH_3O), 3.84 (s, CH_3O of Ar) 6.69 (s, CH-4), 6.94 (d, J = 8.8 Hz, 2 x CH of Ar), 7.22 (s, CH-1), 7.45 (d, J = 8.8 Hz, 2 x CH of Ar); ^{13}C NMR ($CDCl_3$) δ: 11.5, 23.0, 26.5, 27.3, 27.8, 29.8, 38.0, 38.7, 43.2, 43.9, 50.3, 55.3, 55.6, 57.9, 90.8, 111.5, 113.4 (2C), 127.8, 127.9, 130.5 (2C), 131.2, 132.5, 136.7, 154.3, 158.5; HRMS for $C_{27}H_{35}O_3$ $[M + H]^+$: calc 407.25807, found 407.25980; HPLC purity: 95.6 %.

5. Description of the enzymatic assay (EROD assay)

The inhibitory activity of tested compounds against CYP1B1 was determined using the ethoxyresorufin-*O*-deethylase (EROD) assay according to the manufacturer's instructions (Corning, Woburn, MA, USA; BD Bioscience, Mississauga, ON, Canada). Briefly, 7-ethyl-*O*-resorufin (4 μM) was used as enzyme substrate and a NADPH regenerating system containing 1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 3.3 mM $MgCl_2$ (solution A) and 0.5 U/mL glucose-6-phosphate dehydrogenase (solution B) was used instead of NADPH (1.67 mM) in a final volume of 90 μL in tris-acetate buffer pH 7.4 by well. Each compound was dissolved in DMSO and added (5 μL) to the incubation mixture to obtain the final concentration needed. The DMSO concentration in the well was adjusted to 0.1%. Recombinant human CYP1B1 equipped with P450 reductase (Supersomes; BD Bioscience) was used as enzyme source and the enzymatic reaction in triplicate was initiated by adding 5 μL of CYP1B1 (0.7 pmol) dissolved in tris-acetate buffer. The plate was incubated for 45 min at 37 °C under slight agitation and fluorescence derived from the formation of resorufin was recorded (96-well microplate reader INFINITE 500 PRO series; Tecan, Männedorf, Switzerland) with excitation

and emission filters at 535 and 590 nm, respectively. For the screening, the percentage of inhibition was calculated at one concentration (3 μM), whereas several concentrations (0.01, 0.05, 0.1, 0.5, 1.0, 3.0, and 5.0 μM) were used for the IC_{50} value determined using GraphPad Prism 6 software.

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