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Article

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Targeting Cytochrome P450 (CYP) 1B1 Enzyme with Four Series of A-ring Substituted Estrane Derivatives: Design, Synthesis, Inhibitory Activity and Selectivity

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ABSTRACT: Cytochrome P450 (CYP) 1B1 is involved in the bioactivation of procarcinogens and drug-resistance. To obtain selective CYP1B1 inhibitors overs CYP1A1, we synthesized four series of estrane derivatives: *1)* twelve estrone (E1)- and 17β-estradiol (E2)-derivatives bearing a 3- or a 4-pyridinyl core at C2, C3, or C4, *2)* eight estrane derivatives with different sulfur groups at C3, *3)* nineteen E1- and E2-derivatives bearing distinct aryls at C2, and *4)* five D-ring derivatives. E2-derivatives were more active than oxidized E1-analogs, thus highlighting the key role of 17β-OH for interaction with CYP1B1. 2-(4-Fluorophenyl)-E2 was the best CYP1B1 inhibitor (IC_{50} = 0.24 μM) with a selectivity index (SI) of 20 over CYP1A1. Furthermore, the addition of a C17α-ethynyl group as D-ring modification improved the SI to 25 with only a slight loss of activity (IC_{50} = 0.37 μM). Our docking results showed that these compounds fit better into the CYP1B1 binding site than that of CYP1A1.

KEYWORDS: Steroid, estrane derivatives, cytochrome P450, CYP1B1, enzyme inhibitor, molecular docking

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 is involved in the bioactivation of several procarcinogens such as benzo[α]pyrene, 2) it catalyzes the 4-hydroxylation of 17β-estradiol (E2) subsequently leading to the formation of E2-3,4-quinone, a mutagenic compound able to bind DNA covalently; and 3) it is associated with drug-resistance because it interacts with the metabolism of some anticancer agents, such as docetaxel, paclitaxel, and cisplatin.[5-11] Furthermore, an overexpression of CYP1B1 has been observed in distinct types of human cancers, such as breast, lung, esophagus, skin, testis, colon, lymph node, and brain. Therefore, the use of a CYP1B1 inhibitor associated with an anticancer agent could be a promising strategy to treat cancer cases in which CYP1B1 is overexpressed, and has developed drug resistance.[12,13]

In a recently published review,[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 for their ability to interact with CYP1 enzymes and the best CYP1B1 inhibitors known to date belong to these two families of chemicals.[13-16] However, only two studies were conducted for CYP1B1 inhibition by steroid derivatives.[17,18] This is relatively surprising, given that some estrane-based derivatives are substrates of this enzyme. Having developed an expertise in the synthesis of steroid derivatives,[19-24] we therefore decided to focus on this family of natural products, especially the C18-, C19- and

C21-steroids, as potential CYP1B1 inhibitors. In an initial study, the inhibitory activity of a collection of 90 steroid derivatives previously synthesized in our laboratory was evaluated on CYP1B1 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 in this series of steroid derivatives.[25] In this respect, through molecular modeling studies, we observed 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 CYP1B1 inhibitory activity could be improved by introducing, a chemical group (pyridine, triazole, etc.) on the A-ring of the C18-steroid core, known to interact with heme systems.[26-30]

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 which was co-crystallized with CYP1B1.[31] In this 3D-structure, the phenyl moiety of ANF is oriented towards CYP1B1's heme system iron atom. By introducing different chemical groups on the phenyl moiety of ANF, Cui *et al.* 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 1C**).

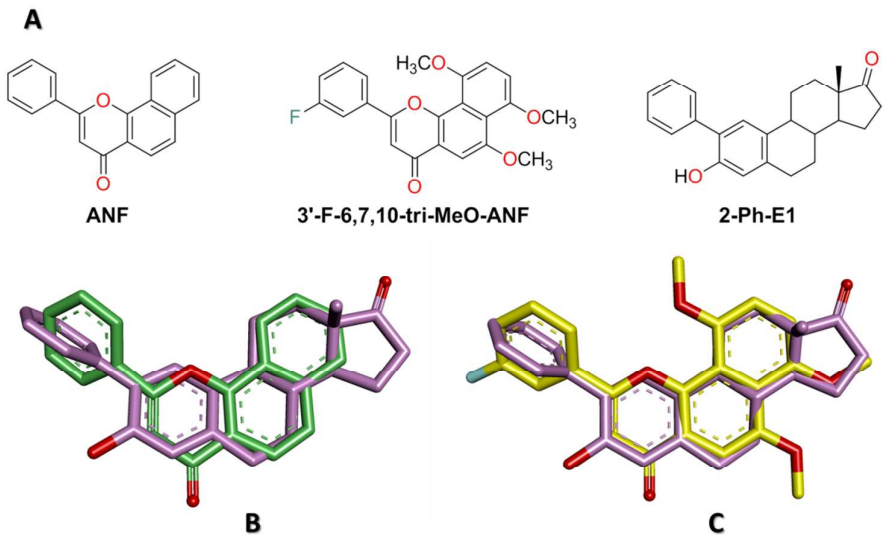


Figure 1. (**A**) Chemical structures of α-naphthoflavone (ANF), 3'-F-6,7,10-tri-MeO-ANF and 2-Ph-E1; (**B**) Molecular overlay of ANF (*green*) and 2-Ph-E1 (*purple*) cores; (**C**) Molecular overlay of 3'-F-6,7,10-tri-MeO-ANF (*yellow*) and 2-Ph-E1 (*purple*) cores. The molecular overlays, as well as the images, were produced using the BIOVIA Discovery Studio Visualizer.[32] The molecular geometry of compounds was previously optimized by the semi-empirical PM6 method using Gaussian 09 software.[33]

Based on these observations and on docking studies, we synthesized three series of estrane derivatives in order to identify potent CYP1B1 inhibitors (**Figure 2**). Series 1 regroups twelve E1 and E2 derivatives bearing a 3- or a 4-pyridinyl moiety at C2, C3 or C4 of the estrane core. Indeed, the nitrogen atom of pyridine is known to generate interactions with the iron of heme systems.[26] Some results obtained with this series of pyridinyl-estrane derivatives have been the subject of a preliminary report by our research group.[34] It was observed that estrane derivatives bearing the pyridinyl moiety at C2 were much more potent CYP1B1 inhibitors than those with a pyridinyl at C3 or C4. Moreover, compounds bearing a 17β-OH exhibited better inhibitory effect than their oxidized form (17-C=O). The best two

CYP1B1 inhibitors of series 1 were the E2-derivative with a 3-pyridinyl at C2 (compound **1b**) and its counterpart with a 4-pyridinyl at C2 (compound **2b**).

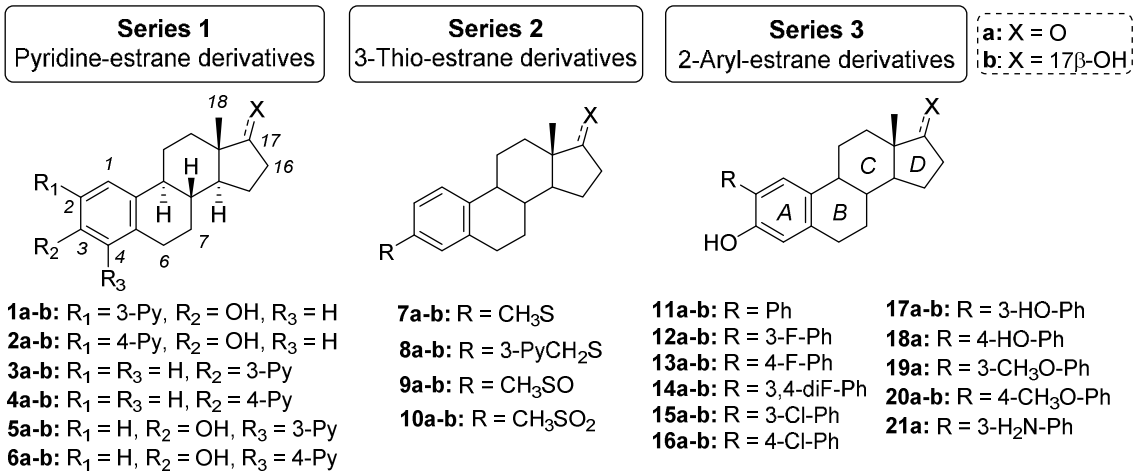


Figure 2. Chemical structures of the three series of estrane derivatives designed and synthesized for the inhibition of CYP1B1. Partial numbering of carbons (left structure) and steroid (A-D) ring identification (right structure) are reported.

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 series 2, a sulfur group was introduced at C3 to promote an interaction with the heme group. In series 3, the design of E1 and E2 derivatives was based on the similarity of estrane nuclei 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 the metabolization of the 17β-OH group and to see the impact on inhibitory activity. Selectivity of the inhibitors for CYP1B1 over CYP1A1 was also assessed.

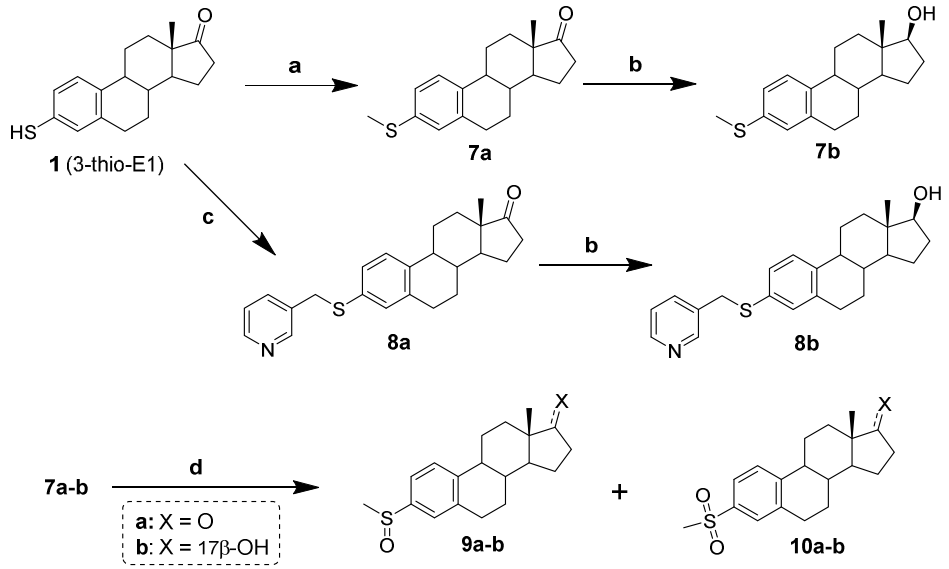
RESULTS AND DISCUSSION

Synthesis of new estrane derivatives

Synthesis of estrane-sulfur derivatives (Series 2)

Based on the results of a first screening that identified 3-thio-E1 as a weak CYP1B1 inhibitor,[25] we synthesized eight new estrane derivatives bearing different sulfur groups at C3 (compounds **7a-b**, **8a-b**, **9a-b**, and **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 the presence of cesium carbonate (Cs_2CO_3) in refluxing acetonitrile (ACN). Thereafter, compound **7b** was obtained from **7a** by a reduction of the C17 ketone with sodium borohydride ($NaBH_4$) at 0 °C. For this kind of unsubstituted D-ring steroid, the angular 18-CH_3 group provides an excellent stereoselectivity and only the 17-alcohol was observed.[35-37]

Scheme 1[a]



[a] 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-ylmethylthio group at C3 of the steroid core. Since pyridine is known to generate interactions with heme sytems,[26] we synthesized these compounds for a combined effect of the sulfur atom and the pyridine moiety. Compound **8a** was obtained from 3-(bromomethyl)pyridine hydrobromide in refluxing ACN with Cs_2CO_3 and triethylamine (TEA). A small quantity 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 $NaBH_4$.

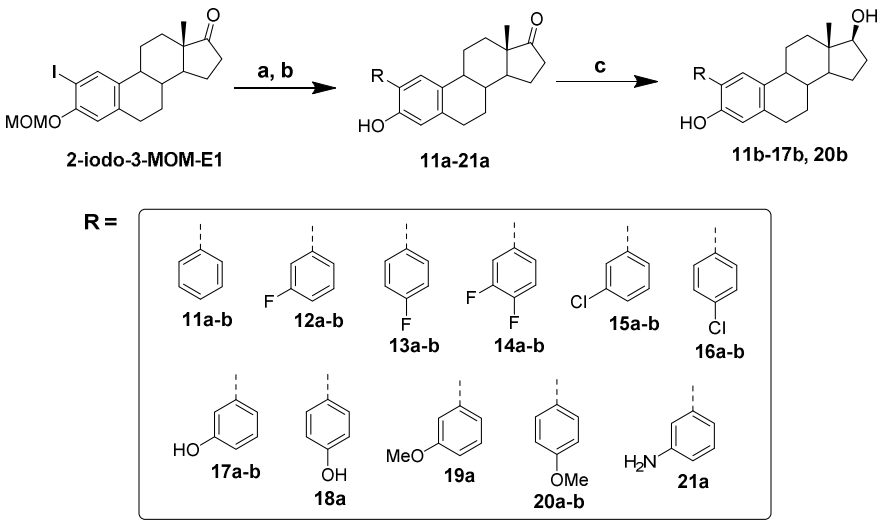
Finally, we synthesized the sulfoxides **9a-b** and the sulfones **10a-b** because they are highly probable metabolites of **7a-b** due to the oxidizability of the sulfur atom. Compounds **9a** and **10a** were obtained in the same time from **7a** using potassium peroxymonosulfate (Oxone) as oxidizing agent. This reaction was performed in MeOH-H_2O at 0 °C and was 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** instead of **7a** as starting product.

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] when introducing small groups to the phenyl moiety of ANF, we developed a third series of estrane derivatives with 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. Fluorine or chlorine atom was chosen in the design of **12a-b**, **13a-b**, **14a-b**, **15a-b**, and **16a-b** because ANF derivatives bearing halogens on the phenyl core were found to be particulary 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 increase the selectivity of inhibition towards CYP1B1.[13,16] The aniline derivative **21a** was also prepared based on the potential 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 structure-activity relationship (SAR) study. Finally, 2-phenyl-estrane derivatives **11a-b** were added as references for this series of 2-aryl-estrane derivatives.

Scheme 2[a]



[a] Chemical synthesis of the 2-aryl-estrane derivatives (Series 3). Reagents and conditions: (**a**) R-$B(OH)_2$, $Pd(dppf)Cl_2$, K_3PO_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.

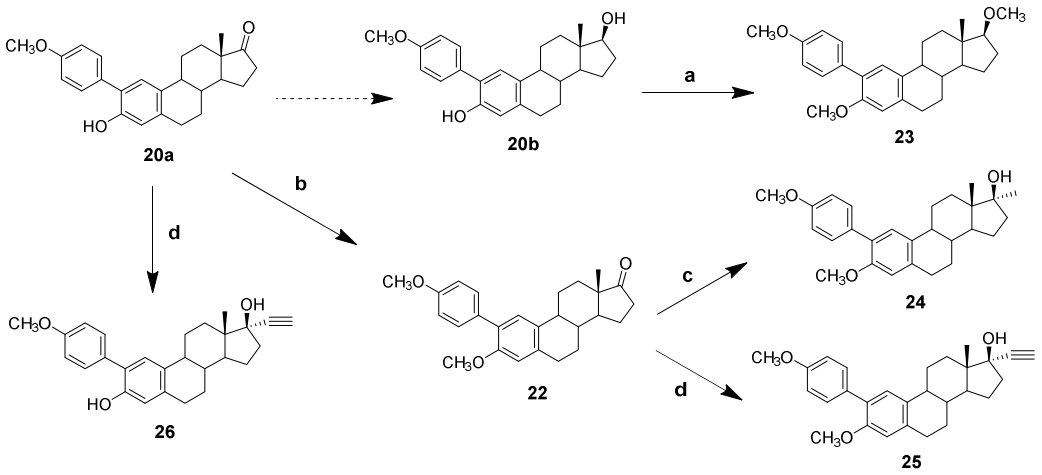
The nineteen estrane derivatives of series 3 (compounds **11a-b** to **21a**) were synthesized by reacting 2-iodo-3-methoxymethyl (MOM) ether-E1 with the corresponding arylboronic acid in the same reaction conditions used for the synthesis of the series of

pyridinyl-estrane derivatives (Series 1). Reactions were performed at 120 °C in dimethylformamide (DMF) under microwave irradiation with potassium phosphate tribasic (K_3PO_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 presence of the MOM protecting group at C3 of the steroid backbone was important. In fact, this ether group promotes the reaction by complexing the palladium catalyst. After the Suzuki coupling, the MOM group was hydrolysed using chlorhydric acid in methanol (MeOH) 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**.

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

In our preliminary report on pyridinyl-estrane derivatives (Series 1),[34] we evaluated the plasma 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 towards an oxidation by Phase-I metabolism enzymes as well as a glucuronidation or a sulfatation by Phase-II metabolism enzymes, we synthesized D-ring derivatives **23-26** (**Scheme 3**). The potent CYP1B1 inhibitor **20b** (Series 3) was thus selected and modified by adding a small methyl, ethynyl or methoxy group known to protect a 17β-OH group.

Scheme 3[a]



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

Compound **23** was synthesized from **20b** by a di-*O*-methylation of 3- and 17β-OH groups, with methyl iodide in anhydrous DMF, and sodium hydride as base. 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 was next used for the preparation of **24** and **25**. Compound **24** was obtained by adding a methyl group at position 17α of **22** using methylmagnesium iodide in anhydrous toluene. Compound **25** 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. Finally, compound **26** was obtained using the same reaction conditions reported for the synthesis of **25**, but with **20a** as starting product. This compound was prepared to evaluate the impact of the methoxy group at C3 on the metabolic stability of these D-ring derivatives.

Assessment of estrane derivatives as CYP1B1 inhibitors

The inhibitory activities on CYP1B1 of 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 CYP1B1 activity. ANF, a known potent CYP1B1 inhibitor, was used as reference for these EROD assays.[15,31]

CYP1B1 inhibitory activities of estrane-sulfur derivatives

Concerning the EROD assay results obtained with the eight thio-estrane derivatives as CYP1B1 inhibitors (**Table 1**), only **8a-b** have shown 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 17β-OH for CYP1B1 inhibition and is in agreement with the results obtained with the compounds of series 1.[34] On the other hand, compounds **7a-b**, with a thiomethyl group at C3 and their likely metabolites resulting from the oxidation of the sulfur atom, compounds **9a-b** and **10a-b**, are very weak inhibitors. Considering these results, the sulfur atom does not seem to play a major role in 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**. It should be noted that they are less potent inhibitors than ANF (48 ± 3, 61 ± 3, and 94 ± 3% of inhibition at 0.3 μM for **8a**, **8b**, and ANF, respectively).

Table 1. Inhibition of CYP1B1 and CYP1A1 activities by a series of estrane-sulfur derivatives (Series 2)

Compd[a]	C3-substituent	17-O or 17β-OH	CYP1B1 Inhibition (%) at 0.3 μM[b]	CYP1B1 Inhibition (%) at 3 μM[b]	CYP1A1 Inhibition (%) at 0.3 μM[b]	CYP1A1 Inhibition (%) at 3 μM[b]
7a	CH_3S	O	14 ± 3	34 ± 3	--	--
7b	CH_3S	17β-OH	5 ± 1	26 ± 5	--	--
8a	3-PyrCH_2S	O	**48 ± 3**	**87 ± 1**	**9 ± 9**	**36 ± 5**
8b	3-PyrCH_2S	17β-OH	**61 ± 3**	**94 ± 1**	**11 ± 2**	**40 ± 8**
9a	CH_3SO	O	16 ± 3	57 ± 1	--	--
9b	CH_3SO	17β-OH	10 ± 2	25 ± 2	--	--
10a	CH_3SO_2	O	7 ± 4	7 ± 1	--	--
10b	CH_3SO_2	17β-OH	0 ± 3	5 ± 3	--	--
ANF	-	-	94 ± 3	101 ± 1	30 ± 3	75 ± 6

[a] See **Scheme 1** for the structure of these compounds.

[b] Inhibition of the transformation of resorufin ethyl ether into resorufin by human CYP1B1 or CYP1A1 in the presence of NADPH. Two experiments performed in triplicate (±SD).

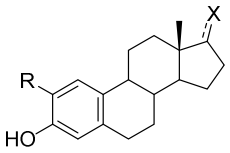
CYP1B1 inhibitory activities of 2-aryl-estrane derivatives

The results obtained with compounds from 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 toward 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 that are 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, a 3-methoxyphenyl, and

a 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 moiety at position 2. Thus, the introduction of a C2-phenyl ring bearing small chemical groups is a good strategy to optimize the inhibition of estrane derivatives towards CYP1B1.

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



Compd[a]	R	X	CYP1B1 Inhibition (%) at 0.3 µM[b]	CYP1B1 Inhibition (%) at 3 µM[b]	CYP1A1 Inhibition (%) at 0.3 µM[b]	CYP1A1 Inhibition (%) at 3 µM[b]
1a	3-Py	O	38 ± 2[c]	90 ± 2	--	--
1b	3-Py	17β-OH	85 ± 1[c]	100 ± 1	53 ± 4	77 ± 2
2a	4-Py	O	21 ± 3[c]	55 ± 11	--	--
2b	4-Py	17β-OH	**87 ± 1**[c]	**102 ± 1**	**5 ± 4**	**14 ± 11**
11a	Ph	O	69 ± 3	101 ± 2	5 ± 5	16 ± 2
11b	Ph	17β-OH	82 ± 2	101 ± 1	9 ± 10	45 ± 1
12a	3-F-Ph	O	67 ± 7	101 ± 1	3 ± 2	23 ± 3
12b	3-F-Ph	17β-OH	88 ± 1	101 ± 1	12 ± 5	48 ± 7
13a	4-F-Ph	O	71 ± 2	101 ± 1	2 ± 1	8 ± 1
13b	4-F-Ph	17β-OH	**91 ± 2**	**103 ± 1**	**3 ± 1**	**0 ± 4**

14a	3,4-di-F-Ph	O	77 ± 2	100 ± 1	1 ± 1	15 ± 20
14b	3,4-di-F-Ph	17β-OH	**92 ± 2**	100 ± 1	2 ± 1	47 ± 23
15a	3-Cl-Ph	O	74 ± 1	100 ± 2	5 ± 4	11 ± 4
15b	3-Cl-Ph	17β-OH	**91 ± 1**	100 ± 1	16 ± 8	51 ± 13
16a	4-Cl-Ph	O	79 ± 2	101 ± 2	9 ± 10	5 ± 5
16b	4-Cl-Ph	17β-OH	**96 ± 2**	99 ± 3	12 ± 10	63 ± 6
17a	3-HO-Ph	O	**92 ± 2**	103 ± 1	10 ± 1	39 ± 13
17b	3-HO-Ph	17β-OH	**96 ± 1**	100 ± 1	10 ± 3	57 ± 3
18a	4-HO-Ph	O	19 ± 1	30 ± 2	--	--
19a	3-MeO-Ph	O	48 ± 4.5	81 ± 1	--	--
20a	4-MeO-Ph	O	**95 ± 2**	103 ± 1	8 ± 10	18 ± 9
20b	4-MeO-Ph	17β-OH	**97 ± 1**	100 ± 2	14 ± 7	51 ± 12
21a	3-H_2N-Ph	O	36 ± 4	55 ± 3	--	--
ANF	-	-	91 ± 2	104 ± 1	30 ± 3	75 ± 6

[a] See **Scheme 2** and **Figure 2** for the structures of these compounds.

[b] Inhibition of the transformation of resorufin ethyl ether into resorufin by human CYP1B1 or CYP1A1 in the presence of NADPH. Two or three experiments performed in triplicate (±SD).

[c] Data from reference 34.

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, thus confirming 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 (91 ± 2% of inhibition at 0.3 μM). Compound **13b**, with a 4-fluorophenyl core at C2, had the same CYP1B1 inhibitory profile as its analog, compound **14b**, with a 3,4-difluorophenyl moiety (91 ± 2 and 92 ± 2% 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 (91 ± 1 and 96 ± 2% 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.

Compounds **17a** and **17b** are the E1- and E2-derivatives with a 3-hydroxyphenyl core at C2 (92 ± 2 and 96 ± 1% of CYP1B1 inhibition at 0.3 μM, respectively). Similarly, **20a** and **20b** are 4-methoxyphenyl derivatives (95 ± 2 and 97 ± 1% of inhibition at 0.3 μM, respectively). If we compare the results obtained for the compounds from series 3, these four chemicals show higher inhibition percentages than those 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 ± 1 and 97 ± 1% 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 (**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.

CYP1B1 inhibitory activities of D-ring derivatives (Series 4)

The formation of a 17β-methoxy derivative (compound **23**) or a tertiary alcohol (compounds **24-26**) is a known strategy to stabilize the steroidal C17-secondary alcohol or C17-ketone towards Phase I and Phase II metabolism. The impact of these small modifications on CYP1B1 inhibition was consequently assessed by testing their inhibitory activities on CYP1B1 (**Table 3**). The results showed that the 17β-OMe derivative **23** did not inhibit CYP1B1 (20% at 3 μM), whereas the 17α-methylation and 17α-ethynylation (compounds **24** and **25**, respectively) were less detrimental for the inhibition of CYP1B1 (46 and 55% at 3 μM, respectively). However, the presence of a MeO group at C3 instead of the OH group, a change that would have been helpful to stabilize the phenolic A-ring, also reduced the inhibitory potency at 0.3 μM from 77 to 38% for **20a** and **22**, respectively. A dual modification at C3 (*O*-methylation) and C17α (ethynylation) was detrimental to CYP1B1 inhibition, but the 17α-ethynylation alone of compound **20b**, producing the tertiary alcohol **26**, allowed to keep very good levels of enzyme inhibition (58% and 94% at 0.3 and 3 μM, respectively).

Table 3. Inhibition of CYP1B1 activity by D-ring derivatives (Series 4)

Compd[a]	C2-	C3-	C17β/17α-	CYP1B1	CYP1B1	CYP1A1	CYP1A1

	Substituent	Substituent	Substituent	Inhibition (%) at 0.3 µM[b]	Inhibition (%) at 3 µM[b]	Inhibition (%) at 0.3 µM[b]	Inhibition (%) at 3 µM[b]
23	4-CH_3O-Ph	CH_3O	OCH_3 / H	11 ± 4	20 ± 1	1 ± 1	5 ± 6
24	4-CH_3O-Ph	CH_3O	OH / CH_3	8 ± 8	46 ± 7	2 ± 1	6 ± 4
25	4-CH_3O-Ph	CH_3O	OH / C≡CH	6 ± 3	55 ± 2	4 ± 3	9 ± 1
26	4-CH_3O-Ph	HO	OH / C≡CH	58 ± 1	94 ± 1	7 ± 5	23 ± 3
22	4-CH_3O-Ph	CH_3O	= O	38 ± 1	79 ± 1	1.2[c]	4.7[c]
20a	4-CH_3O-Ph	HO	= O	77 ± 1	85 ± 2	8 ± 10	18 ± 9
20b	4-CH_3O-Ph	HO	OH / H	97 ± 1	107 ± 1	14 ± 7.2	51 ± 12
ANF	--	--	--	93 ± 1	104 ± 1	30 ± 3	75 ± 6

[a] See **Schemes 1** and **3** for the structures of these compounds.

[b] Inhibition of the transformation of resorufin ethyl ether into resorufin by human CYP1B1 or CYP1A1 in the presence of NADPH. Two experiments performed in triplicate (±SD).

[c] One experiment performed in triplicate.

Assessment of estrane derivatives as CYP1A1 inhibitors

CYP1A1 is mainly associated with the formation of 2-OH-E2 contrary to CYP1B1, which is mainly involved in the formation of mutagenic 4-OH-E2. Therefore, to reduce the ratio 4-OH-E2 / 2-OH-E2, a CYP1B1 inhibitor should ideally not inhibit CYP1A1. The most potent CYP1B1 inhibitors from series 1 (compounds **1b** and **2b**), series 2 (compounds **8a** and **8b**), series 3 (compounds **11a-b** to **17a-b** and **20a-b**) and D-ring derivatives (compounds **23-26**) were tested as CYP1A1 inhibitors using the EROD assay (**Tables 1-3**). The only differences with the CYP1B1 assay were the use of less CYP1A1 (0.13 pmol instead of 0.7 pmol) and a shorter reaction time (25 min instead of 45 min). As reported in **Table 1**, the best

CYP1B1 inhibitor of series 2, the estrane-sulfur derivative **8b**, showed a moderate selectivity of inhibition for CYP1B1 over CYP1A1, as similarly observed for ANF. The keto derivative **8a** provided better selectivity, but this compound only weakly inhibited CYP1B1. In the compounds of series 1, the 4-pyridinyl derivative **2b** is more selective for CYP1B1 than the 3-pyridinyl derivative **1b** (**Table 2**). For series 3, sixteen of the nineteen aryl-estrane derivatives were tested and found to be selective for CYP1B1 over CYP1A1. At the higher concentrations tested (3 μM), their inhibition levels ranged from 1 to 78% for CYP1A1, whereas they fully inhibited CYP1B1, thus suggesting a very good selectivity for most of these 2-aryl-estrane derivatives. The most selective compound from series 3 is clearly the 4-fluoro-phenyl derivative **13b**, which did not significantly inhibit CYP1A1 at 0.3 and 3 μM. As reported in **Table 3**, compounds with a D-ring modification inhibited weakly CYP1A1, but they are also weak CYP1B1 inhibitors. The ethynylated compound **26** was however selected because it provided the best compromise between inhibition of CYP1B1 and non-inhibition of CYP1A1.

Selectivity of CYP1B1 inhibition over CYP1A1

The results obtained from the screening of estrane derivatives on both CYP1B1 and CYP1A1 allowed us to select the best representative compounds of series 1-4. Compounds **2b**, **8b**, **13b**, and **26** were thus tested at several concentrations, and the IC_{50} values were determined from the curve reported in **Figures 3** and **4**. The IC_{50} values ranged from 0.24 to 0.54 μM for CYP1B1 and from 3.4 to 9.2 μM for CYP1A1, which values provided a good to very good selectivity index (SI) ranging from 6 to 25 (**Table 4**). The estrane-sulfur derivative **8b** is the less potent and selective CYP1B1 inhibitor, as suggested by the screening results. The 4-pyridinyl- and 4-fluoro-phenyl-estrane derivatives **2b** and **13b** produced the same inhibition of CYP1B1, but **13b** is more selective (SI = 16 and 20, respectively). Interestingly,

compound **26,** with a D-ring modification (17α-ethynyl), provided the best selectivity (SI = 25) for CYP1B1 among these four compounds, and it is also more selective than the reference ANF (SI = 11).

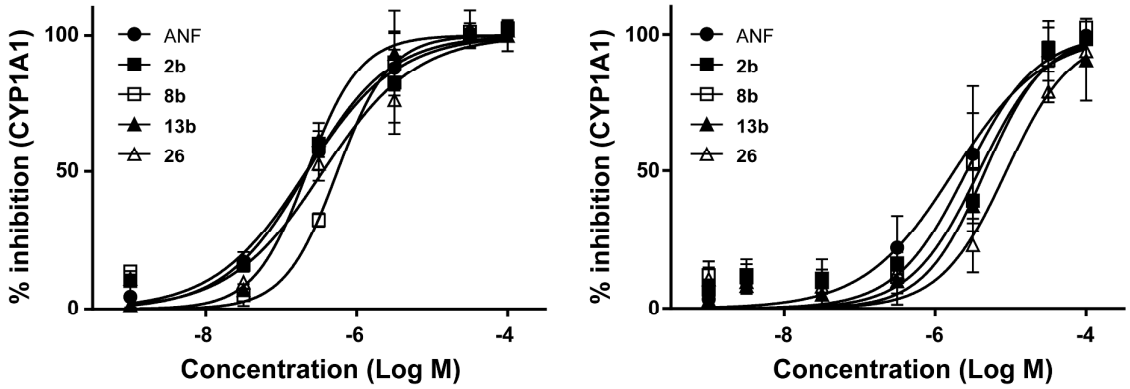


Figure 3. Curves of CYP1B1 (**A**) and CYP1A1 (**B**) inhibition obtained with E2 derivatives **2b**, **8b**, **13b**, and **25**. Each curve is representative of two experiments performed in triplicate.

Table 4. Selectivity of the inhibition for CYP1B1 over CYP1A1

Series	Cpd	Name	CYP1B1 IC_{50} (μM)[a]	CYP1A1 IC_{50} (μM)[a]	SI[b]
1	**2b**	2-(4-pyridinyl)-E2	0.24 ± 0.05	3.9 ± 1.9	16
2	**8b**	3-(pyridine-3-ylmethanethio)-E2	0.54 ± 0.08	3.4 ± 2.6	6
3	**13b**	2-(4-fluoro-phenyl)-E2	0.24 ± 0.04	4.9 ± 0.6	20
4	**26**	2-(4-methoxy-phenyl)-17α-ethynyl-E2	0.37 ± 0.09	9.2 ± 3.5	25
--	ANF	nonsteroidal inhibitor	0.24 ± 0.08	2.7 ± 2.3	11

[a] Selectivity index (SI) = IC_{50} (CYP1A1) / IC_{50} (CYP1B1).

[b] Two experiments performed in triplicate (±SEM)

Docking results

To investigate the potential binding modes of **2b**, **8b**, **13b**, and **26,** as well as their selectivity, we carried out docking simulations using GOLD 5.4 software and the X-ray structure of CYP1B1 (PDB ID: 3PM0) and CYP1A1 (PDB ID: 4I8V). For comparison purposes, the structure of ANF was also docked. The first interesting observation for the molecules screened in this study (**Table 5**) is that they displayed better GoldScore (GS) and ChemPLP fitness score (CFS) for CYP1B1 than CYP1A1 (except for **8b**). For instance, the best docked conformation of ANF fit better into the binding site of CYP1B1 (GS = 69.8 and CFS = 95.5) than CYP1A1 (GS = 69.6 and CFS = 87.1). These results showed the same tendency as our experimental data against both isoenzymes.

Table 5. Docking scores for 2b, 8b, 13b, and 26 at the binding site of CYP1B1 and CYP1A1 enzymes [a]

Compound	CYP1B1		CYP1A1	
	GS	CFS	GS	CFS
2b	54.5	89.4	46.2	90.7
8b	64.2	102.2	63.7	108.2
13b	56.7	91.7	40.1	75.3
26	22.6	96.0	16.3	65.7
ANF	69.8	95.5	69.6	87.1

[a] For GoldScore (GS) and ChemPLP fitness score (CFS) definitions, see Supporting Information.

The binding mode of **2b** into CYP1B1 (GS = 54.5 and CFS = 89.4) and CYP1A1 (GS = 46.2 and CFS = 90.7) differs considerably, since the steroidal scaffold is inversely oriented for each enzyme (**Figure S1**, Supporting Information). However, it is important to note that the pyridine ring at C2 is oriented towards the heme group without forming nitrogen-iron bonds as performed by **1b**.[34] The most important interactions of **2b** with the binding site of CYP1B1 include: an H-bond of 17β-OH with Asn-228 (distance = 1.7 Å); pi-stacking interactions with residues Phe-134, Phe-231 and Gly-329; a pi-cation interaction between the pyridine ring and the iron atom of the heme group; as well as weak hydrophobic bonds with Leu-509, Phe-268, Ala-133 and Ala-330. On the contrary, the docked structure of **2b** in CYP1A1 interacts by means of: two H-bonds between 17β-OH and 3-OH with Ser-116 (distance = 1.7 Å) and Asp-320 (distance = 2.0 Å), respectively; pi-interactions with Phe-224, Gly-316 and the heme group as well. Nevertheless, we found that the orientation of 18-CH_3 of **2b** promotes clashing into both enzymes, suggesting that this group may disrupt the correct binding of this derivative. However, we assume that **2b** fit better into the binding site of CYP1B1 because there is just one bump with Gln-332, instead of two formed with residues of Ile-115 and Phe-224 into the cavity of CYP1A1.

Unlike **2b**, the binding mode of **8b** showed the same orientation into the active sites of CYP1B1 (GS = 64.2 and GFS = 102.2) and CYP1A1 (GS = 63.7 and 108.2) (**Figure S2**, Supporting Information). The 17β-OH group for both **8b** structures is positioned to perform H-bonds with Asn-265 (distance = 2.6 Å) and Asn-255 (distance = 2.0 Å) for CYP1B1 and CYP1A1, respectively. Moreover, these structures form pi-interactions with the side chain of residues Phe and Gly as well as hydrophobic contacts with residues Ala, Leu and Val. Surprisingly, **8b** produced a pi-interaction between its A-ring and the aromatic ring of Phe-231 into the cavity of CYP1B1, whereas this interaction is absent with CYP1A1 because Phe-

224 residue is more distanced from the A-ring of **8b**. In addition, as observed for **2b**, the 18-CH_3 group of **8b** exhibited steric hindrance with the aliphatic side chain of Ile-115 into CYP1A1, but this was not the case with CYP1B1. Thus, **8b** fits better into CYP1B1, providing slighty high GS than that obtained for CYP1A1.

As observed for **8b**, the steroidal core of **13b** is positioned in the same way at the binding site of both enzymes (**Figure 4**). Interestingly, we found improved GS, as well as CFS scores for CYP1B1 (GS = 56.7 and CFS = 91.7) than CYP1A1 (GS = 40.1 and CFS = 75.3), suggesting better selectivity. The 4-fluorophenyl ring attached to C2 of **13b** is oriented towards the heme group into the cavity of both isoenzymes. Curiously, **13b** possesses the same binding mode than that found with pyridinyl-estrane CYP1B1 inhibitors **2b** and **1b**.[34] According to our docking results, **13b** produces H-bonds between 17β-OH and Asn residues such as Asn-228 (distance = 1.8 Å) and Asn-222 (distance = 1.9 Å) into the binding site of CYP1B1 and CYP1A1, respectively. Furthermore, the steroidal core interacts with hydrophobic moieties of Val, Ala and Phe. Conversely, since the aromatic ring at C2 of **13b** is closer to the iron atom of CYP1A1 than CYP1B1, we found that this group promotes pi-cation interactions with the heme group of CYP1A1 instead of pi-pi T-shaped interactions with the pyrrole subunits of porphyrin as performed into CYP1B1. Nevertheless, we believe that **13b** exibited better GS and CFS for CYP1B1 because its A-ring produces more pi-interactions in comparison with CYP1A1. These include pi-pi T-shaped with Phe-231 and Pi-sigma with Ala-330.

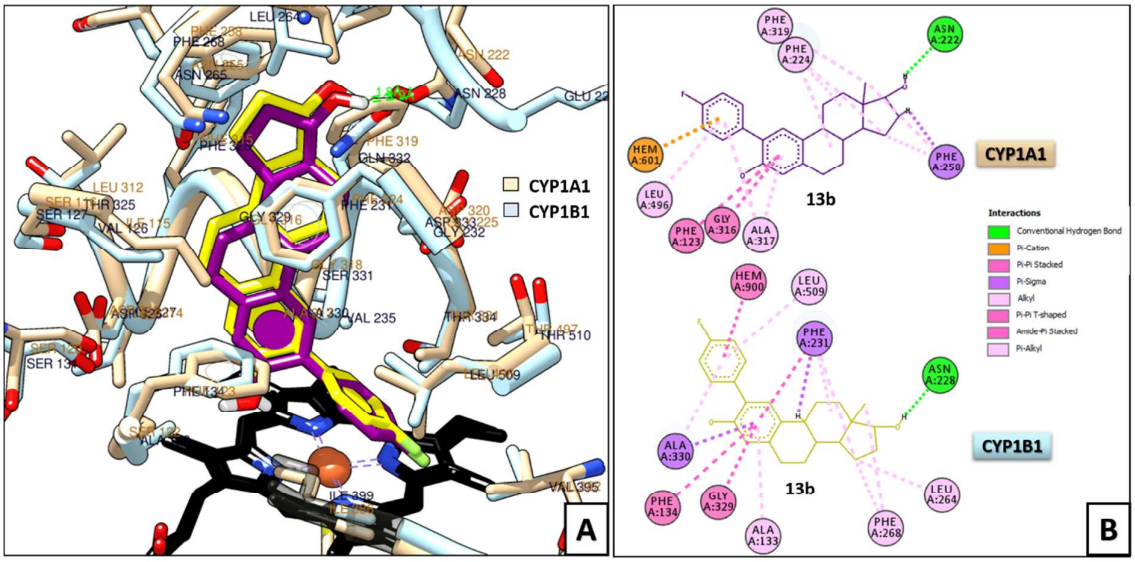


Figure 4. (A) Binding mode of **13b** docked at the binding site of CYP1B1 (light blue) (PDB 1D: 3PMO) and CYP1A1 (khaki) (PDB 1D: 418V). Yellow sticks represent **13b** docked into the active site cavity of CYP1B1, while purple sticks represent **13b** docked into the active site cavity of CYP1A1. Black sticks represent the heme group for both enzymes, whereas the iron atom is highlighted as a red sphere. **(B)** 2D diagram of docked **13b** in CYP1B1 and CYP1A1 showing the interactions with the residues at the binding site.

The binding mode of **26** is oriented oppositely to its counterparts **2b**, **8b**, and **13b** (Figure 5). For instance, the 17β-OH group is oriented towards the heme group instead of forming H-bonds with Asn residues. On the other hand, the steroid core of **26** displays hydrophobic contacts with Phe, Ile and Ala residues, as well as the heme group. In addition, the A-ring performs pi-interactions with Phe and Gly residues. Moreover, the aromatic ring at C2 is oriented towards the side chain of Phe-268 and Phe-258 residues of CYP1B1 and CYP1A1, respectively, forming pi-pi T-shaped interactions. Interestingly, the 18-CH_3 of **26** has a negative interaction with Leu-496 into CYP1A1, whereas a CH of the methoxyphenyl moiety at C2 bumps with Leu-264 into CYP1B1. Unexpectedly, the 3-OH group of **26** forms a H-bond (distance = 2.4 Å) with the amide residu of Asn-265 into the binding site of

CYP1B1 but not with Asn-255 of CYP1A1. Therefore, this key interaction promotes a better fit into the active site cavity of CYP1B1, and is the main reason why we found much better GS (22.6) and CFS (96.0) than those of CYP1A1 (GS = 16.3 and CFS = 65.7). In fact, the experimental data showed that CYP1B1 inhibition was notably improved by replacing the 3-CH_3O group of **25** by the 3-OH of **26**, thus supporting that the H-bond donor group of **26** may interact with Asn-265. It is worth mentioning that, since the C17α-ethynyl moiety is a rigid group, it did not allow the correct interaction of the 17β-OH group with Asn-228 residue as performed by **2b** and **13b**. Thus, we assume that this group promotes the inversed orientation of **26**, also giving a slightly negative impact in its inhibitory effect against CYP1B1.

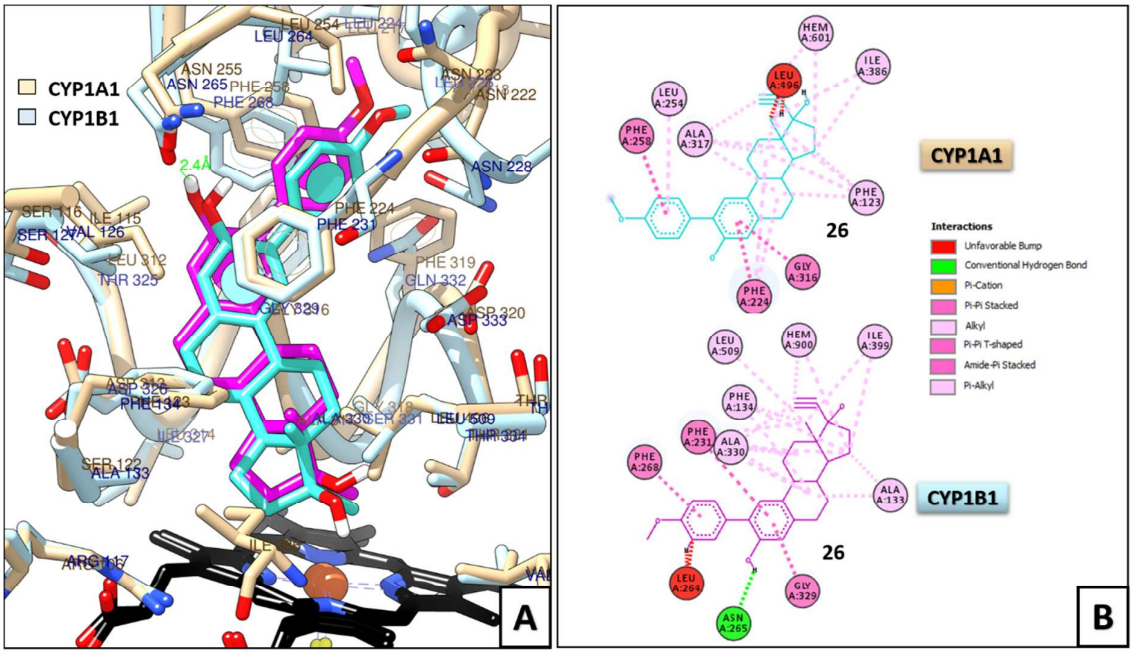


Figure 5. (A) Binding mode of **26** docked at the binding site of CYP1B1 (light blue) (PDB 1D: 3PMO) and CYP1A1 (khaki) (PDB 1D: 418V). Magenta sticks represent **26** docked into the active site cavity of CYP1B1 while cyan sticks represent **26** docked into the active site cavity of CYP1A1. Black sticks represent the heme group for both enzymes, whereas the iron atom is highlighted as a red

sphere. **(B)** 2D diagram of docked **26** in CYP1B1 and CYP1A1, showing the interactions with the residues at the binding site.

CONCLUSION

We synthesized four series of new estrane derivatives in order to assess their inhibitory activity towards CYP1B1 and CYP1A1: 1) twelve E1 and E2 derivatives bearing a 3- or 4-pyridinyl core at positions 2, 3 or 4 of the steroid nucleus (Series 1), eight estrane derivatives with different sulfur groups at C3 (Series 2), nineteen 2-aryl-estrane derivatives (Series 3), and four D-ring derivatives (Series 4). These steroid derivatives were obtained in good to very good yields, and were fully characterized by IR, ^{1}H NMR, ^{13}C NMR, and HRMS analyses.

The results obtained with series 1 highlighted a very interesting correlation between docking studies and the inhibitory activities measured for this series of pyridinyl-estrane derivatives, showing that position 2 is the best for CYP1B1 inhibition. With its 4-pyridinyl group at C2, compound **2b** is a potent and selective CYP1B1 inhibitor. Compounds of series 2 were found to be less potent CYP1B1 inhibitors among the 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.

The design of the compounds in series 3 was based on the superimposition of ANF and the steroid core of E1 and E2. The results were very promising, because almost all the 2-aryl-estrane derivatives were potent CYP1B1 inhibitors. Thus, position 2 of the estrane nucleus appears to be the best one to introduce chemical groups such as aryl and pyridinyl moieties. The most potent CYP1B1 inhibitors of this series are compounds **13b** and **20b**, bearing a 4-fluorophenyl and a 4-methoxyphenyl group at C2, respectively. As observed for

series 1 and 2, compounds of series 3 with a hydroxy function at C17 (17β-OH) were more potent CYP1B1 inhibitors than their oxidized homologs (ketone at C17). This conclusion highlights the key role of 17β-OH for CYP1B1 inhibition by the formation of an H-bond between 17β-OH and the Asn-228 residue. This secondary alcohol (17β-OH) was also stabilized against the metabolism by adding a 17α-ethynyl group, a D-ring modification that increased the selective inhibition of CYP1B1 over CYP1A1, as exemplified by compound **26**, but induced an inversion of its binding mode, contrary to other estrane derivatives **2b**, **8b**, and **13b**.

Our docking results showed that these new estrane derivatives fit better into the CYP1B1 binding site than that of CYP1A1; correlating with our experimental data. Moreover, we found that Asn residues play a critical role by forming H-bonds with the 17β-OH and 3-OH of the estrane derivatives into the active site cavity of both isoenzymes. However, pi-interactions and unfavorable bumps with the steroid core are also crucial to the inhibitory activity and selectivity of these novel steroid derivatives. Finally, it should be noted that a small chemical modification in the structure of these estrane derivatives is enough to induce a different orientation of the compound into the catalytic site of CYP1B1 or CYP1A1.

EXPERIMENTAL SECTION

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 (Montreal, 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. All final compounds showed a purity ≥ 95% (95.2 – 99.9%; 23 compounds) except for compounds **7a**, **8a**, **9b**, **12a-b**, **15a-b**, **16b**, and **21a** (89.5 – 94.6%, 9 compounds). Infrared (IR) spectra were recorded on a MB 3000 ABB FTIR spectrometer (Quebec, 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 source. High-resolution mass spectra (HRMS) were provided by Pierre Audet at the Chemistry Department of Université Laval (Quebec, QC, Canada).

Synthesis of estrane-sulfur derivatives (Series 2: compounds 7a,b to 10a,b)

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 **1a** 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%.

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%.

3-(Pyridin-3-ylmethanethio)-estra-1,3,5(10)-triene-17-one (8a)

To a solution of 3-thioestrone (300 mg, 1.05 mmol), Cs_2CO_3 (1.37 g, 4.2 mmol) and tetrabutylammonium iodide (TBAI) (416 mg, 1.12 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/MeOH (97:3) as eluent to give compound **8a** as a light yellow solid (138 mg, 35%). 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 = 8.5 Hz, J_2 = 18.8 Hz, 16β-CH), 2.84 (m, CH_2-6), 4.04 (s, CH_2S), 7.03 (d, J = 1.7 Hz, CH-4), 7.08 (dd, J_1 = 8.1 Hz, J_2 = 1.9 Hz, CH-2), 7.21 (m, CH-1 and CH of Pyr), 7.62 (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, 123.4 (vw), 126.1, 128.3, 131.4, 131.8, 133.9 (vw), 136.3, 137.4, 139.0, 148.3, 149.8, 220.6; HRMS for $C_{24}H_{28}NOS$ $[M + H]^+$: calc 378.18861, found 378.18819; HPLC purity: 93.5%.

3-(Pyridin-3-ylmethanethio)-estra-1,3,5(10)-trien-17β-ol (8b)

To a solution of compound **8a** (65 mg, 0.17 mmol) in MeOH/DCM 9:1 (10 mL) was added under argon atmosphere and at 0 °C $NaBH_4$ (3.0 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 (43 mg, 66%). 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), 2.78 (m, CH_2-6), 3.72 (d, J = 8.5 Hz, 17α-CH), 4.03 (s, CH_2S), 7.01 (s, CH-4), 7.06 (dd, J_1 = 8.1 Hz, J_2 = 1.7 Hz, CH-2), 7.18 (d, J = 9.0 Hz, CH-1), 7.21 (m, CH of Pyr), 7.61 (dt, J_1 = 7.8 Hz, J_2 = 1.9 Hz, CH of Pyr), 8.43 (d, J = 1.9 Hz, CH of Pyr), 8.46 (dd, J_1 = 4.8 Hz, J_2 = 1.6

Hz, CH of Pyr); ^{13}C NMR ($CDCl_3$) δ: 11.0, 23.1, 26.0, 27.0, 29.3, 30.5, 36.7, 36.8, 38.4, 43.2, 44.2, 50.1, 81.7, 123.3, 126.1, 128.2, 131.5 (2C), 133.8, 136.3, 137.7, 139.7, 148.3, 149.9; HRMS for $C_{24}H_{30}NOS$ $[M + H]^+$: calc 380.20426, found 380.20343; HPLC purity: 97.0%.

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%.

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%.

2-Aryl-estrane derivatives (Series 3: 11a,b to 21a)

2-Phenyl-estra-1,3,5(10)-triene-17-one (11a)

To a solution of 2-iodo-3-methoxymethylether(MOM)-estrone(E1)[34] (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%.

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%.

2-(3-Fluorophenyl)-estra-1,3,5(10)-triene-17-one (12a)

To a solution of 2-iodo-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%.

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%.

2-(4-Fluorophenyl)-estra-1,3,5(10)-triene-17-one (13a)

To a solution of 2-iodo-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%.

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%.

2-(3,4-Difluorophenyl)-estra-1,3,5(10)-triene-17-one (14a)

To a solution of 2-iodo-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) *v*: 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%.

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%.

2-(3-Chlorophenyl)-estra-1,3,5(10)-triene-17-one (15a)

To a solution of 2-iodo-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%.

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%.

2-(4-Chlorophenyl)-estra-1,3,5(10)-triene-17-one (16a)

To a solution of 2-iodo-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%.

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%.

2-(3-Hydroxyphenyl)-estra-1,3,5(10)-triene-17-one (17a)

To a solution of 2-iodo-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%.

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%.

2-(4-Hydroxyphenyl)-estra-1,3,5(10)-triene-17-one (18a)

To a solution of 2-iodo-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%.

2-(3-Methoxyphenyl)-estra-1,3,5(10)-triene-17-one (19a)

To a solution of 2-iodo-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%.

2-(4-Methoxyphenyl)-estra-1,3,5(10)-triene-17-one (20a)

To a solution of 2-iodo-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%.

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%.

2-(3-Aminophenyl)-estra-1,3,5(10)-triene-17-one (21a)

To a solution of 2-iodo-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%.

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

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); HRMS for $C_{26}H_{31}O_3$ $[M + H]^+$: calc 391.2268, found 391.2296. HPLC purity: 99.2%.

2-(4-Methoxyphenyl)-3,17β-dimethoxy-estra-1,3,5(10)-trien-17β-ol (23)

To a solution of compound **20b** (30 mg, 0.08 mmol) in anhydrous DMF (5 mL) were added under argon atmosphere sodium hydride (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 **23** 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%.

2-(4-Methoxyphenyl)-3-methoxy-17α-methyl-estra-1,3,5(10)-trien-17β-ol (24)

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 (CH_3MgI) (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 **24** as a white amorphous solid (65 mg, 78%). IR (KBr) *v*:

3433 (OH); ^{1}H 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%.

2-(4-Methoxyphenyl)-3-methoxy-17α-ethynyl-estra-1,3,5(10)-trien-17β-ol (25)

To a solution of trimethylsilylacetylene (0.1 mL, 0.80 mmol) (4 eq.) in anhydrous diethyl ether (6 mL) under argon atmosphere was added methyl lithium (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 **25** 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; HRMS for $C_{28}H_{33}O_3$ $[M + H]^+$: calc 417.2424, found 417.2427; HPLC purity: 97.7%.

2-(4-Methoxyphenyl)-17α-ethynyl-estra-1,3,5(10)-trien-17β-ol (26)

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 and 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 **26** 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%.

Description of the enzymatic assays (EROD assay) for CYP1B1 and CYP1A1

The inhibitory activity of tested compounds against CYP1B1 or CYP1A1 was determined using the ethoxyresorufin-*O*-deethylase (EROD) assay according to 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 or CYP1A1 equipped with P450 reductase (Supersomes; BD Bioscience) was used as enzyme source and the enzymatic reaction was initiated in triplicate by adding 5 μL of CYP1B1 (0.7 pmol) or CYP1A1 (0.13 pmol) dissolved in tris-acetate buffer. The plate was incubated for 45 min (CYP1B1) or 25 min (CYP1A1) 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} values determined using GraphPad Prism 6 software.

Docking methodology

Docking studies were performed using GOLD-5.4 software,[38] and the X-ray structures of CYP1B1 (PDB ID: 3PM0) and CYP1A1 (PDB ID: 4I8V). The chemical structures of E2 and ANF were retrieved from the ZINC database.[39] From these structures, all ligands were built by systematic modifications and then energy-minimized by semi-empirical PM6 method

using Gaussian 09 software.[33] Docking simulations were carried out within a 10 Å radius of the co-crystallized molecule, using the following parameters: 100 GA runs per molecule and 125,000 operations. ChemPLP fitness score was chosen as scoring function, whereas GOLD score was selected as re-score function within the goldscore_p450_csd template. The dockings were ranked according to the value of the GOLD score and ChemPLP fitness score function; only the best ranked solution for each ligand was included in further analysis. The ligand interactions were analyzed using GOLD-5.4 software along with BIOVIA Discovery Studio Visualizer.[32] Figures and 2D diagrams were produced with UCSF Chimera[40] program and BIOVIA Discovery Studio Visualizer 2017, respectively.

ASSOCIATED CONTENT

Supporting Information

The supporting information is available free of charge on the ACS Publication website at …….. 1) Docking score definitions and 2D diagrams of **2b** and **8b** docked in CYP1B1 and CYP1A1. 2) ^{1}H NMR, ^{13}C NMR, HRMS, and HPLC spectra for all compounds. 3) Molecular formula strings.

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Notes

The authors declare no competing interests.

ABBREVIATIONS

ACN, acetonitrile; ANF, α-naphthoflavone, CFS, ChemPLP fitness score; dppf, 1,1'-bis(diphenylphosphino)ferrocene; E1, estrone; E2, estradiol; EROD, ethoxyresorufin-*O*-deethylase; GS, gold score; PLP, piecewise linear potential; SD, standard diviation; SEM, standard error of the mean; SI, selectivity index; TEA, trimethylamine; TBAI, terabutylammonium iodide.

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REFERENCES

(1) Bruno, R. D.; Njar, V. C. O. Targeting cytochrome P450 enzymes: A new approach in anti cancer drug development. *Bioorg. Med. Chem.*, **2007**, *15*, 5047-5060.

(2) Danielson, P. B. The cytochrome P450 superfamily: Biochemistry, evolution and drug metabolism in humans. *Curr. Drug Metab.*, **2002**, *3*, 561-597.

(3) Nebert, D. W.; Wikvall, K.; Miller, W. L. Human cytochromes P450 in health and disease. *Phil. Trans. R. Soc. B.*, **2013**, *368*, 20120431.

(4) Nelson, D.R.; The cytochrome P450 homepage, *Hum. Genomics*, **2009**, *4*, 59-65.

(5) Hayes, C. L.; Spink, D. C.; Spink, B. C.; Cao, J. Q.; Walker, N. J.; Sutter, T. R. 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 9776-9781.

(6) Lee, A. J.; Cai, M. X.; Thomas, P. E.; Conney, A. H.; Zhu, B. T. Characterization of the oxidative metabolites of 17beta-estradiol and estrone formed by 15 selectively expressed human cytochrome p450 isoforms. *Endocrinology*, **2003**, *144*, 3382-3398.

(7) Cavalieri, E. L.; Stack, D. E.; Devanesan, P. D.; Todorovic, R.; Dwivedy, I.; Higginbotham, S.; Johansson, S. L.; Patil, K. D.; Gross, M. L.; Gooden, J. K.; Ramanathan, R.; Cerny, R. L.; Rogan, E. G. Molecular origin of cancer: Catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 10937-10942.

(8) Go, R.E.; Hwang, K.A.; Choi, K.C.; Cytochrome P450 1 family and cancers, *J. Steroid Biochem. Mol. Biol.*, **2015**, *147*, 24-40.

(9) McFadyen, M. C. E.; McLeod, H. L.; Jackson, F. C.; Melvin, W. T.; Doehmer, J.; Murray, G. I. Cytochrome P450 CYP1B1 protein expression : A novel mechanism of anticancer drug resistance. *Biochem. Pharmacol.*, **2001**, *62*, 207-212.

(10) Rochat, B.; Morsman, J. M.; Murray, G. I.; Figg, W. D.; McLeod, H. L. Human CYP1B1 and anticancer agent metabolism: mechanism for tumor-specific drug inactivation? *J. Pharmacol. Exp. Ther.*, **2001**, *296*, 537-541.

(11) Cui, J.; Meng, Q.; Zhang, X.; Cui, Q.; Zhou, W.; Li, S. Design and synthesis of new α-naphthoflavones as cytochrome P450 (CYP) 1B1 inhibitors to overcome docetaxel-resistance associated with CYP1B1 overexpression. *J. Med. Chem.*, **2015**, *58*, 3534-3547.

(12) Murray, G. I.; Taylor, M. C.; McFadyen, M. C.; McKay, J. A.; Greenlee, W. F.; Burke, M. D.; Melvin, W. T. Tumor-specific expression of cytochrome P450 CYP1B1. *Cancer Res.*, **1997**, *57*, 3026-3031.

(13) Cui, J.; Li, S. Inhibitors and prodrugs targeting CYP1: a novel approach in cancer prevention and therapy. *Curr. Med. Chem.*, **2014**, *21*, 519-552.

(14) Dutour, R.; Poirier, D.; Inhibitors of cytochrome P450 (CYP) 1B1. *Eur. J. Med. Chem.*, **2017**, *135*, 296-306.

(15) Chun, Y. J.; Kim, S. Discovery of cytochrome P450 1B1 inhibitors as new promising anti-cancer agents. *Med. Res. Rev.*, **2003**, *23*, 657-668.

(16) Dong, J.; Zhang, Q.; Cui, Q.; Huang, G.; Pan, X.; Li, S. Flavonoids and naphthoflavonoids: wider roles in the modulation of cytochrome P450 family 1 enzymes. *ChemMedChem*, **2016**, *11*, 2102-2118.

(17) Dawling, S.; Roodi, N.; Parl, F. F. Methoxyestrogens exert feedback inhibition on cytochrome P450 1A1 and 1B1. *Cancer Res.*, **2003**, *63*, 3127-3132.

(18) Ciolino, H. P.; MacDonald, C. J.; Yeh, G. C. Inhibition of carcinogen-activating enzymes by 16α-fluoro-5-androsten-17-one. *Cancer Res.*, **2002**, *62*, 3685-3690.

(19) Poirier, D. Contribution to the development of inhibitors of 17β-hydroxysteroid dehydrogenase types 1 and 7: key tools for studying and treatingestrogen-dependent diseases. *J. Steroid Biochem. Mol. Biol.*, **2011**, *125*, 83-94.

(20) Laplante, Y.; Cadot, C.; Fournier, M. A.; Poirier, D. Estradiol and estrone C-16 derivatives as inhibitors of type 1 17β-hydroxysteroid dehydrogenase: blocking of ER^+ breast cancer cell proliferation induced by estrone. *Bioorg. Med. Chem.*, **2008**, *16*, 1849-1860.

(21) Maltais, R.; Fournier, M. A.; Poirier, D. Development of 3-substituted-androsterone derivatives as potent inhibitors of 17β-hydroxysteroid dehydrogenase type 3. *Bioorg. Med. Chem.*, **2011**, *19*, 4652-4668.

(22) Maltais, R.; Ayan, D.; Trottier, A.; Barbeau, X.; Lagüe, P.; Bouchard, J. E.; Poirier, D. Discovery of a non-estrogenic irreversible inhibitor of 17β-hydroxysteroid dehydrogenase type 1 from 3-substituted-16β-(*m*-carbamoylbenzyl)-estradiol derivatives. *J. Med. Chem.*, **2014**, *57,* 204-222.

(23) Boivin, R. P.; Luu-The, V.; Lachance, R.; Labrie, F.; Poirier, D. Structure-activity relationships of 17α-derivatives of estradiol as inhibitors of steroid sulfatase. *J. Med. Chem.*, **2000**, *43,* 4465-4478.

(24) Ayan, D.; Maltais, R.; Hospital, A.; Poirier, D. Chemical synthesis, cytotoxicity, selectivity and bioavailability of 5α-androstane-3α,17β-diol derivatives. *Bioorg. Med. Chem*. **2014**, *22*, 5847-5859.

(25) Poirier, D.; Roy, J.; Cortés-Benítez, F.; Dutour, R. Targeting cytochrome P450 (CYP) 1B1 with steroid derivatives. *Bioorg. Med. Chem. Lett.*, **2016**, *26*, 5272-5276.

(26) DeVore, N. M.; Scott, E. E. Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK-001. *Nature*, **2012**, *482*, 116-119.

(27) Njar, V.C.O.; Brodie, A.M.H. Inhibitors of 17α-hydroxylase/17,20-lyase (CYP17): Potential agents for the treatment of prostate cancer. *Curr. Pharm. Design*, **1999**, *5*, 163-180.

(28) Salvador, J.A.R.; Pinto, R.M.A.; Silvestre, S.M. Steroidal 5α-reductase and 17α-hydroxylase/17,20-lyase (CYP) inhibitors useful in the treatment of prostatic diseases. *J. Steroid Biochem. Mol. Biol.*, **2013**, *137*, 199-222.

(29) Adhikari, N.; Amin, S.A.; Saha, A.; Jha, T. Combating breast cancer with non-steroidal aromatase inhibitors (NSAIs): Understanding the chemico-biological interactions through comparative SAR/QSAR study. *Eur. J. Med. Chem.*, **2017**, *137*, 365-438.

(30) Rahman, M.N.; Vlahakis, J.Z.; Roman, G.; Vukomanovic, D.; Szarek, W.A.; Nakatsu, K.; Jia, Z. Structural characterisation of human heme oxygenase-1 in complex with azole based-inhibitors. *J. Inorg. Biochem.*, **2010**, *104*, 324-330.

(31) Wang, A.; Savas, U.; Stout, C. D.; Johnson, E. F. Structural characterization of the complex between α-naphthoflavone and human cytochrome P450 1B1. *J. Biol. Chem*., **2011**, *286*, 5736-5743.

(32) Dassault Systèmes BIOVIA, Discovery Studio, version 17.2.0.16349, San Diego: Dassault Systemes, **2016**.

(33) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T., ; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.;

Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian 09, Revision E.01; Gaussian Inc: Wallingford, **2009**.

(34) Dutour, R.; Cortés-Benítez, F.; Roy, J.; Poirier, D. Structure-based design and synthesis of new estrane-pyridine derivatives as cytochrome P450 (CYP) 1B1 inhibitors. ACS *Med. Chem. Lett.*, **2017**, *8*, 1159-1164.

(35) Wheeler, D. M. S.; Wheeler, M. M. In *Organic Reactions in Steroid Chemistry*; Fried, J., Edwards, J. A., Eds.; Van Nostrand Reinhold: New York, 1992; pp 61.

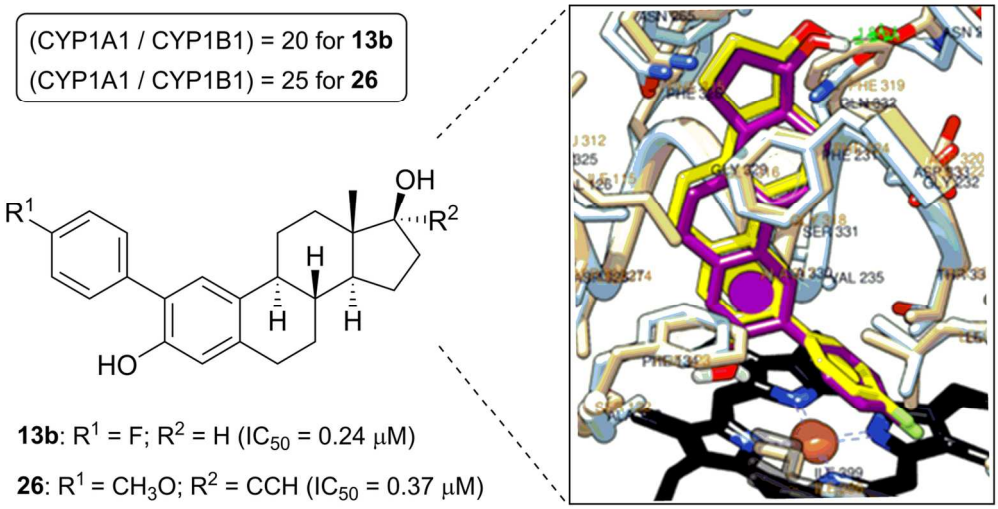
(36) Cadot, C.; Poirier, D.; Philip, A. First synthesis of a steroid containing an unstable 19-nor-androsta-1,5-diene-3-one system. *Tetrahedron*, **2006**, *62*, 4384-4392.

(37) Cadot, C.; Laplante, Y.; Kamal, F.; Luu-The, V.; Poirier, D. C6-(*N*,*N*-butyl-methyl-heptanamide) derivatives of estrone and estradiol as inhibitors of type 1 17β-hydroxysteroid dehydrogenase: chemical synthesis and biological evaluation. *Bioorg. Med. Chem*., **2007**, *15*, 714-726.

(38) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a generic algorithm for flexible docking. *J. Mol. Biol*., **1997**, *267*, 727-748.

(39) Irwin, J. J.; Shoichet, B. K. ZINC − A free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model.,* **2005**, *45*, 177-182.

(40) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera – A visualization system for exploratory research and analysis. *J. Comput. Chem.*, **2004**, *25*, 1605-1612.



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