

IRREVERSIBLE 17 β -HSD1 INHIBITORS**CROSS-REFERENCE TO RELATED APPLICATION**

The present patent application claims the benefits of priority of U.S. Provisional Patent
5 Application No. 63/519,779 entitled "IRREVERSIBLE 17BETA-HSD1 INHIBITORS", and
filed at the United States Patent and Trademark Office on August 15, 2023, the content of
which is incorporated herein by reference.

TECHNICAL FIELD

The present invention relates to the inhibition of 17 β -HSD1. In particular, the present
10 invention provides compounds of formula (I) and compositions comprising thereof, which
provide a potent, non-estrogenic and irreversible inhibitory effect on 17 β -HSD1. The
invention further provides therapeutic methods and uses based on the new compounds and
compositions.

BACKGROUND

15 Estradiol (E2) is produced in premenopausal women mainly in the ovaries. It reaches the
target tissues by endocrine route, where it exerts its effect through interaction with the
estrogen receptor (ER) α . After menopause, the plasma E2 level drops to 1/10 of the E2
level in premenopausal women. E2 is then mainly produced in the peripheral tissue, such
as breast tissue, endometrium, adipose tissue, skin from inactive precursors, etc. These
20 reactions take place with the participation of various steroidogenic enzymes (hydroxysteroid
dehydrogenases, aromatase), in the peripheral tissue, where the active estrogens exert
their effect. As a consequence of this intracrine mechanism for the formation of E2, its
concentration in the peripheral tissue, especially in estrogen-dependent diseases, is higher
than in healthy tissue. In particular, the growth of many cancer cell lines is stimulated by a
25 locally increased E2 concentration. Furthermore, the occurrence and progression of
diseases such as endometriosis, leiomyosis, adenomyosis, menorrhagia, metrorrhagia and
dysmenorrhoea is dependent on a significantly increased E2 level in correspondingly
diseased tissue. Endometriosis is an estrogen-dependent disease affecting approximately
5-10% of all women of childbearing age with 35 - 50% of women suffering abdominal pain
30 u.o. Sterility has signs of endometriosis. This disease is defined as histologically proven
ectopic endometrial glandular and stromal tissue. This chronic disease, which is prone to
recurrences, leads to pain of varying intensity and varying character as well as potentially
to sterility if it is given a corresponding form. Three macroscopic conditions are

distinguished: peritoneal endometriosis, retroperitoneal deep infiltrating endometriosis including adenomyosis uteri and cystic ovarian endometriosis.

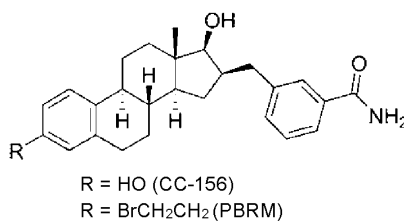
17 β -Hydroxysteroid dehydrogenase type 1 (17 β -HSD1) transforms estrone (E1) into E2, the most potent natural ligand for the ER α . This enzyme also catalyzes the reduction of dehydroepiandrosterone (DHEA) into 5-androstene-3 β ,17 β -diol (A5-diol), a weaker estrogen but especially important after menopause. Inhibitors of 17 β -HSD1 are thus interesting therapeutic agents for the control of estrogen-dependent diseases such as breast cancers and endometriosis.

During the last thirty years, intense efforts were deployed with the goal of designing potent inhibitors of this key steroidogenic enzyme but it is only recently that lead candidates have been reported with very good inhibitory activities. The presence of a residual estrogenic activity associated with steroidal inhibitors, which are often built around an estrane scaffold represented a major drawback in their development.

16 β -(*m*-carbamoylbenzyl)estradiol (CC-156) has been reported as a potent inhibitor of 17 β -HSD1. Despite of its good inhibitory potency, it was found to stimulate *in vitro* both the MCF-7 and T-47D estrogen-sensitive breast cancer cell lines, thus greatly reducing its therapeutic potential.

Many efforts have been made in order to overcome the undesirable residual estrogenic activity, without negatively affecting the inhibitory effect.

Among the new series of compounds developed in this area, the E2 derivative 3-[[[(16 β ,17 β)-3-(2-bromoethyl)-17-hydroxyestra-1,3,5(10)-trien-16-yl]methyl]benzamide (hereinafter also referred as "PBRM") has been reported as the first non-estrogenic irreversible steroidal inhibitor of 17 β -HSD1 (Trottier A. et al., "Insight into the mode of action and selectivity of PBRM, a covalent steroidal inhibitor of 17 β -hydroxysteroid dehydrogenase type 1", *Biochemical Pharmacology*, 2017, 144, 149–161):

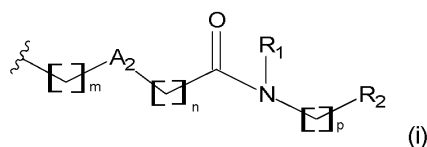


The irreversibility was confirmed by the identification of the covalent binding of PBRM to 17 β -HSD1 (Maltais R. et al., "Discovery of a non-estrogenic irreversible inhibitor of 17 β -hydroxysteroid dehydrogenase type 1 from 3-substituted-16 β -(*m*-carbamoylbenzyl)-estradiol derivatives", 2014, J. Med. Chem., 55(7), 204-222; Li T. et al., "Combined
 5 biophysical chemistry reveals a new covalent inhibitor with low-reactivity alkyl halide", 2018, J. Phys. Chem. Lett. 9, 5275-5280). The irreversible nature of the interaction is especially advantageous with regard to the increased effectiveness and extended action allowed by such a mechanism from a therapeutic perspective.

In spite of the efforts made, however, there is still the need of non-estrogenic irreversible
 10 17 β -HSD1 inhibitors with improved properties.

SUMMARY

The present inventors have surprisingly found that when the PBRM compound was modified by replacing the original 16 β -methylbenzamide moiety by another amide moiety of formula (i):



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the resulting compound not only retained the irreversibility and non-estrogenicity nature of the original PBRM but that a remarkable improvement both in the inhibitory effect on 17 β -HSD1 as well as in metabolic stability was achieved.

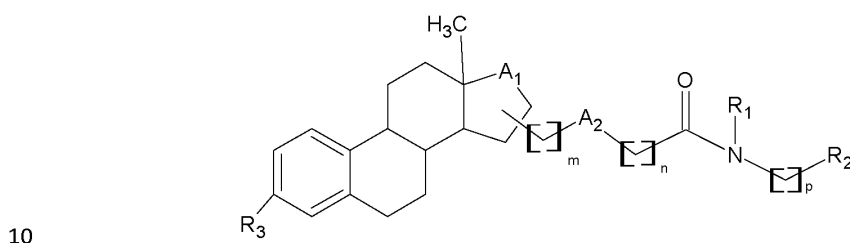
On one hand, Fig. 1 shows that when one of the compounds of the invention (PBRM-II)
 20 was administered to an ER+ breast cancer cell line T-47D, there was a remarkable reduction in the cell growth with respect the control. This is indicative that the compounds of the invention are efficient in the treatment of an estrogen-dependent disease, which is characterized by being associated to high levels of estrogens, but also, and importantly, that they do not "add" estrogenicity to the environment. This is a further indicium of how
 25 safe the compounds of the invention are when they include the amide moiety of formula (i). Contrary, for instance, to CC-156 (a reversible 17 β -HSD1 inhibitor) which, when it is administered to the cells, it induces estrogenicity and gives rise to an increase in the cell growth of the tumoral cells, even when used at low concentration (see Fig. 1).

Looking at Fig. 1 it can also be derived that the compounds of the invention not only inhibit
 30 more potently the target, but that this higher potency translates into a more efficient treatment of the disease at the lowest doses (0.1 and 1 μ M) if compared to PBRM.

On the other hand, Table 1 below shows that the IC₅₀ value was 26-fold reduced when PBRM was modified by replacing the methylbenzamide by an amide moiety of formula (i) located at position 15 β and R₂ being a thiazolyl ring. Furthermore, Fig. 2 shows that the stability of the compound was increased more than 30%.

- 5 Altogether, the compounds provided by the present invention means a great advance in the field of irreversible 17 β -HSD1 inhibitors, in terms of inhibitory potency, efficiency, and safety.

Thus, the present invention provides in a first aspect a compound of formula (I), a stereoisomer or salt thereof:



wherein:

A₁ is selected from C(O) and CHR_{Z1};

A₂ is selected from CH₂ and O;

- 15 R₁ is selected from the group consisting of: hydrogen, (C₁-C₅)alkyl optionally substituted with one or more Z substituents, (C₁-C₅)alkoxy optionally substituted with one or more Z substituents, (C₂-C₅)alkenyl optionally substituted with one or more Z substituents, (C₂-C₅)alkynyl optionally substituted with one or more Z substituents; (C₃-C₈)cycloalkyl; aryl; and heteroaryl;

- 20 R₂ is a heterocyclic aromatic ring having 5, 6 or 7 members selected from the group consisting of: CR_{Z2}, N, S, and O, provided that at least one of the members is N, S or O;

R₃ is (C₁-C₅)alkyl substituted with one or more Br, Br⁷⁶; I, I¹²³, I¹²⁴, or I¹³¹;

R_{Z1} is selected from OH, NR_{X1}R_{X2}, =N-OR_{X3}, halogen, (C₁-C₅)alkoxy, OC(O)(C₁-C₅)alkyl, and OSO₂NR_{X4}NR_{X5};

- 25 R_{Z2} is selected from hydrogen, OH, (C₁-C₁₀)alkyl optionally substituted with one or more Z substituents, and (C₁-C₁₀)alkoxy optionally substituted by one or more Z substituents;

Z is selected from halogen, OH, (C₁-C₅)alkyl, (C₁-C₅)haloalkyl, (C₁-C₅)alkoxy, (C₁-C₅)haloalkoxy, and NR_{x1}R_{x2};

R_{x1}, R_{x2}, R_{x4} and R_{x5} are the same or different and are selected from the group consisting of: hydrogen, (C₁-C₅)alkyl, (C₁-C₅)haloalkyl, (C₁-C₅)alkoxy, and (C₁-C₅)haloalkoxy;

5 R_{x3} is selected from H, and (C₁-C₅)alkyl;

m represents an integer value selected from 0 to 2;

n represents an integer value selected from 0 to 2;

p represents an integer value selected from 0 to 5;

wherein:

10 aryl is an aromatic ring system comprising 5 or 6 CR_c members, wherein R_c is selected from H, halogen, cyano, nitro, (C₁-C₅)alkyl, (C₁-C₅)haloalkyl, -O-(C₁-C₅)alkyl, and -O-(C₁-C₅)haloalkyl; and

heteroaryl is an aromatic ring system comprising 5 or 6 members selected from the group consisting of: CR_d, O, N, NH, and S; wherein R_d is selected from H, halogen, cyano, nitro,

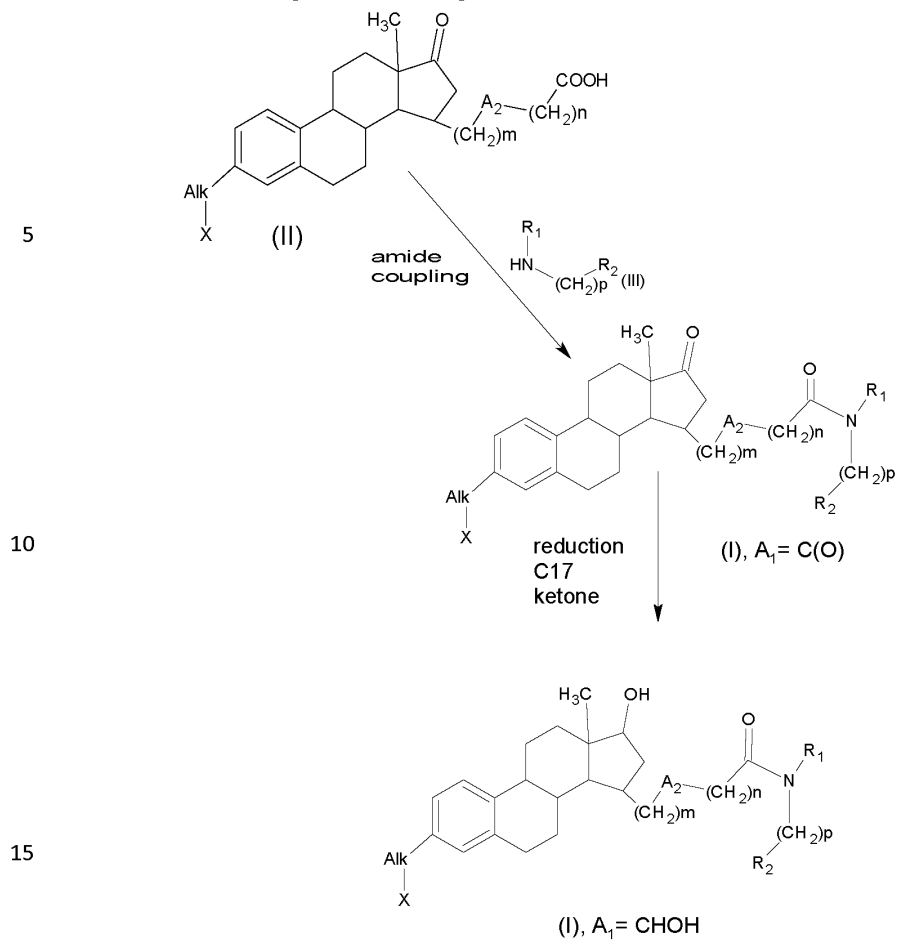
15 (C₁-C₅)alkyl, (C₁-C₅)haloalkyl, -O-(C₁-C₅)alkyl, and -O-(C₁-C₅)haloalkyl; and

wherein the compound of formula (I) has the ability to covalently (i.e., irreversibly) bind to 17β-hydroxysteroid dehydrogenase type 1 enzyme.

The invention also provides synthetic processes for preparing the compounds of the invention.

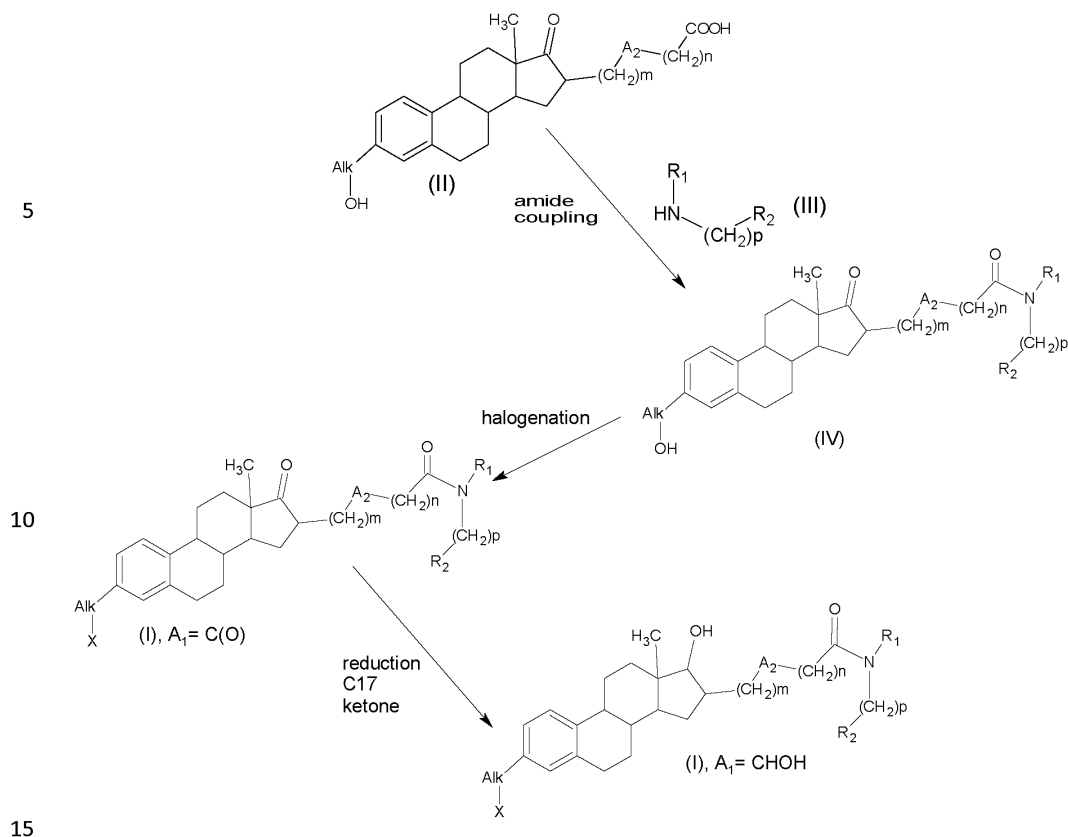
20 Thus, in a second aspect the invention provides a process for preparing a compound of formula (I) as defined in the first aspect of the invention, the process comprising the steps provided in Schemes 1 or 2 below:

Scheme 1: obtaining of C15 analogues



$Alk = (C_1-C_5)alkyl$; $Alk-X = (C_1-C_5)alkyl$ substituted by one X selected from Br, Br^{76} , I, I^{123} , I^{124} , or I^{131} .

Scheme 2: obtaining of C16 analogues



Alk= (C₁-C₅)alkyl; Alk-OH= (C₁-C₅)alkyl substituted by one OH substituent; Alk-X= (C₁-C₅)alkyl substituted by one X selected from Br, Br⁷⁶, I, I¹²³, I¹²⁴, or I¹³¹.

The amide coupling step between the carboxylic acid compound of formula (II) and the amine of formula (III) can be performed using amide coupling agents well-known in the state of the art. Illustrative non-limitative examples of amide coupling agents are independently selected from TBTU, TCTU, HATU, T3P or COMU. The coupling reaction is performed in the presence of DMF or any other suitable aprotic solvent, and an alkali, such as DIPEA.

The halogenation step (either bromination or iodination) of the OH-derivative of formula (IV) can be performed using well-known reagents and conditions. Illustrative non-limitative conditions are provided below, i.e., PPh₃ and CBr₄.

The reduction step of the C17 ketone can also be performed using any suitable reducing reagents and conditions. The reducing agent specifically reduces the ketone and converts it to alcohol. In certain embodiments, specific water-soluble reagents are non-toxic and/or

green reagents. Non-limiting examples of specific water-soluble reducing agents include sodium borohydride (NaBH_4); sodium cyanoborohydride (NaCNBH_3); e.g. nickel (Ni), platinum (Pt) or palladium (Pd in the presence of hydrogen gas (H_2)) catalyst; ammonia borane (H_3NBH_3), borane dimethylamine complex $[(\text{CH}_3)_2\text{NH}\cdot\text{BH}_3]$; borane *tert*-butylamine
5 complex $[(\text{CH}_3)_3\text{CNH}_2\cdot\text{BH}_3]$; or borane-pyrimidine complex.

In a third aspect the present invention provides a pharmaceutical composition comprising the compound as defined in the first aspect of the invention, and one or more pharmaceutically acceptable excipients or carriers.

In a fourth aspect the present invention provides a kit of parts comprising:

- 10 (i) the compound of formula (I) as defined in the first aspect of the invention, and
(ii) a detectable label.

In a fifth aspect the present invention provides a compound as defined in the first aspect of the invention, for use in therapy or diagnosis.

Compounds of the present invention may be useful in therapy, especially in the treatment
15 or prevention of steroid hormone dependent diseases or disorders requiring the inhibition of 17β -HSD1 enzyme, in animals, in particular mammals, and humans. In particular, compounds of formula (I) represent inhibitors of the 17β -HSD1 enzyme, possessing pharmacological properties for the treatment and/or prophylaxis of malignant steroid dependent diseases or disorders such as breast cancer, lung cancer, prostate carcinoma,
20 ovarian cancer, uterine cancer, endometrial cancer and endometrial hyperplasia, but also for the treatment and/or prophylaxis of benign steroid dependent diseases or disorders such as endometriosis, uterine fibroids, uterine leiomyoma, adenomyosis, dysmenorrhea, menorrhagia, metrorrhagia, prostatic hyperplasia, urinary dysfunction, polycystic ovarian syndrome or lower urinary tract syndrome. Further estrogen-dependent
25 diseases which may be treated and/or prevented with an effective amount of a compound of the invention include multiple sclerosis, obesity, rheumatoid arthritis, colon cancer, tissue wounds, skin wrinkles and cataracts.

Thus, in a sixth aspect the present invention provides a method for inhibiting 17β -HSD1 enzyme in a subject, the method comprising the step of administering a therapeutically
30 effective amount of the compound of formula (I) as defined in the first aspect of the invention or of the pharmaceutical composition of the third aspect of the invention, to a subject in need thereof. This aspect can be formulated as a compound of formula (I) as defined in the first aspect of the invention or the pharmaceutical composition of the third aspect of the invention for use in the treatment and/or prevention of a disease by inhibiting the 17β -HSD1

enzyme. This aspect can also be alternatively formulated as the use of a compound of formula (I) as defined in the first aspect of the invention or the pharmaceutical composition of the third aspect of the invention for the manufacture of a medicament for the treatment and/or prevention of a disease by inhibiting the 17 β -HSD1 enzyme.

- 5 In a seventh aspect the present invention provides a method for the treatment and/or prevention of an estrogen-dependent disease, the method comprising the step of administering a therapeutically effective amount of the compound of formula (I) as defined in the first aspect of the invention or the pharmaceutical composition as defined in the third aspect of the invention, to a subject in need thereof. This aspect can alternatively be
10 formulated as the compound of formula (I) as defined in the first aspect of the invention or the pharmaceutical composition of the third aspect of the invention for use in the treatment and/or prevention of an estrogen-dependent disease. This aspect can alternatively be formulated as the use of a compound of formula (I) as defined in the first aspect of the invention or the pharmaceutical composition of the third aspect of the invention for the
15 manufacture of a medicament for the treatment and/or prevention of an estrogen-dependent disease.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1: Effect of 17 β -HSD1 inhibitors on estrogen-dependent T-47D cell proliferation.

- 20 **Fig. 2:** Metabolic stability in human liver microsomes of 17 β -HSD1 inhibitors CC-156 (comparative purpose), PBRM (comparative purpose), and PBRM-II (invention).

Fig. 3: Irreversibility of the compound of the invention PBRM-II.

Fig. 4: Concentration of PBRM-II when given orally (30 mg/kg in DMSO:Sunflower oil/8:92) in mice.

- 25 **Fig. 5A:** Percent of PBRM-II remaining (%PR) versus time (min).

Fig. 5B: Ln of percent of PBRM-II remaining (%PR) versus time (min) and half-life of PBRM-II at 4 μ M in human liver microsomes (HLM).

Fig. 5C: Percent of PBRM remaining (%PR) versus time (min).

- Fig. 5D:** Ln of percent of PBRM remaining (%PR) versus time (min) and half-life of PBRM-II at 4 μ M in human liver microsomes (HLM).
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DETAILED DESCRIPTION

Terms not specifically defined herein should be given the meanings that would be given to them by one of skill in the art in light of the disclosure and the context. As used in the specification, however, unless specified to the contrary, the following terms have the
5 meaning indicated and the following conventions are adhered to.

Throughout the present specification and the accompanying clauses, the words "comprise" and variations such as "comprises", "comprising" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows. The word "comprise" also includes the term
10 "consists of".

For the purposes of the present invention, any ranges given include both the lower and the upper end-points of the range.

In a first aspect the present invention provides irreversible 17 β -HSD1 inhibitors.

In the context of the invention, a compound is a 17 β -HSD1 inhibitor when it reduces the
15 activity of the enzyme. In one embodiment, the compound inhibits at least a 10%, at least a 20%, at least a 30%, at least a 40%, at least a 50%, at least a 60%, at least a 70%, at least a 80% or at least a 90% of the enzymatic activity. There are well-known protocols in the state of the art to determine the inhibitory activity. Illustrative examples are provided below and are based on (a) determining the labelled E1 and E2 after contacting an
20 estrogen-sensitive breast cancer cell line T-47D with [14C]-E1 + cold E1 and the test compound, and (b) calculating the % of transformation and % of inhibition as follows: % transformation = $100 \times \frac{[14C]-E2}{([14C]-E1 + [14C]-E2)}$ and % inhibition = $100 \times \frac{(\% \text{ transformation without inhibitor} - \% \text{ transformation with inhibitor})}{\% \text{ transformation without inhibitor}}$. The concentration of E1 and E2 (labelled and non-labelled) can be determined
25 using any suitable technique. The concentration inhibiting 50% of E1 to E2 transformation (IC₅₀) can be determined any routine protocol or tool, such as using GraphPad Prism 6 software.

In the context of the invention, the irreversibility is due to the ability of the compounds of formula (I) to covalently bind to 17 β -hydroxysteroid dehydrogenase type 1 in an irreversible
30 manner and consequently block the capacity of the enzyme to convert estrone (E1) to estradiol (E2). There are well-known methods in the state of the art to determine whether a compound covalently binds to this enzyme. (Zang et al. Cell Chem Biol., 2019, 26 (11), 1486-1500). Washout experiments, where cells are first exposed to the inhibitor, then

washed out and then allowed to determine the enzymatic activity, are among valuable method in the validation process. The conversion of estrone to estradiol in T-47D cells with washout are compared to "no washout" conditions in presence or not of the inhibitor. The sustained effect to block conversion of estrone to estradiol of covalent inhibitors subjected
5 to the washout experiments is attributed to the irreversible nature of their target engagement. Particular conditions and reagents are provided in the Examples provided below.

In the context of the invention, the term "alkyl" refers to a straight or branched hydrocarbon chain radical containing no unsaturation, and which is attached to the rest of the molecule
10 by a single bond. Typical alkyl groups have from 1 to about 5 carbon atoms, e. g., methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *t*-butyl, and *n*-pentyl, among others.

In the context of the invention, the term "alkenyl" refers to a straight or branched hydrocarbon chain radical containing at least two carbon atoms and at least one C=C double bond, and which is attached to the rest of the molecule by a single bond. Typical
15 alkenyl radicals have from 2 to about 10, 2 to about 8 or 2 to about 6 carbon atoms. In a particular embodiment, the alkenyl group is vinyl, 1-methyl-ethenyl, 1-propenyl, 2-propenyl, or butenyl.

In the context of the invention, the term "alkynyl" refers to a straight or branched hydrocarbon chain radical containing one or more C≡C triple bonds. Examples of alkynyl
20 groups include, but are not limited to, ethynyl, 2-propynyl, and 2-butylnyl.

In the context of the invention, the term "cycloalkyl" refers to a cyclic alkyl, wherein "alkyl" is as defined above. Illustrative non/limitative examples of cycloalkyl are Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl.

25 In the context of the invention, the term "alkoxy" refers to a -O-alkyl, wherein "alkyl" is as defined above. Illustrative non-limitative examples of hydroxyalkyl are methoxy, ethoxy, or *t*-butoxy, among others.

In the context of the invention, the term "haloalkyl" refers to a straight or branched hydrocarbon chain radical containing no unsaturation, wherein one or more of the hydrogen
30 atoms are replaced by halogen. Illustrative non-limitative examples of haloalkyl are chloromethyl, trifluoromethyl, 1-chloro-2-fluoroethyl, and the like.

In the context of the invention, the term "haloalkoxy" refers to a -O-haloalkyl, wherein "haloalkyl" is as defined above.

In the context of the invention, the term "halogen" refers to bromo, chloro, iodo or fluoro.

In the context of the invention, the term "nitro" refers to NO₂.

5 In the context of the invention, the term "cyano" refers to $\text{HC}\equiv\text{N}$.

In the context of the present invention, the term "salt" must be understood as any form of a compound used in accordance with this invention in which said compound is in ionic form or is charged and coupled to a counter-ion (a cation or anion) or is in solution. This definition also includes quaternary ammonium salts and complexes of the active molecule with other
10 molecules and ions, particularly, complexes formed via ionic interactions. The definition includes in particular physiologically acceptable salts; this term must be understood as equivalent to "pharmacologically acceptable salts" or "pharmaceutically acceptable salts".

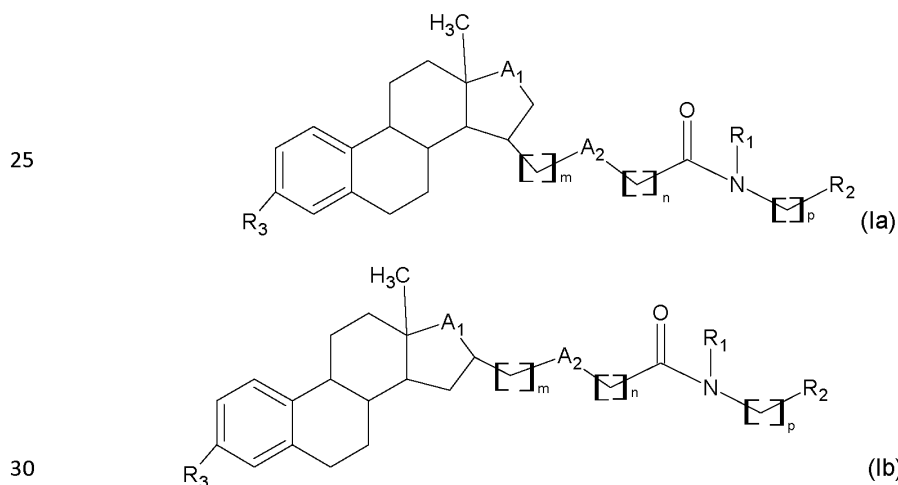
In the context of the present invention, the term "pharmaceutically acceptable salts" means any salt that is tolerated physiologically (normally meaning that it is not toxic, particularly,
15 as a result of the counter-ion) when used in an appropriate manner for a treatment, applied or used, particularly, in humans and/or mammals. These physiologically acceptable salts may be formed with cations or bases and, in the context of this invention, are understood to be salts formed by at least one compound used in accordance with the invention - normally an acid (deprotonated)- such as an anion and at least one physiologically tolerated
20 cation, preferably inorganic, particularly when used in humans and/or mammals. Salts with alkali and alkali earth metals are preferred particularly, as well as those formed with ammonium cations (NH₄⁺). Preferred salts are those formed with (mono) or (di)sodium, (mono) or (di)potassium, magnesium or calcium. These physiologically acceptable salts may also be formed with anions or acids and, in the context of this invention, are understood
25 as being salts formed by at least one compound used in accordance with the invention - normally protonated, for example in nitrogen - such as a cation and at least one physiologically tolerated anion, particularly when used on humans and/or mammals. This definition specifically includes in the context of this invention a salt formed by a physiologically tolerated acid, i.e., salts of a specific active compound with physiologically
30 tolerated organic or inorganic acids - particularly when used on humans and/or mammals. Examples of this type of salts are those formed with: hydrochloric acid, hydrobromic acid,

sulphuric acid, methanesulfonic acid, formic acid, acetic acid, oxalic acid, succinic acid, malic acid, tartaric acid, mandelic acid, fumaric acid, lactic acid or citric acid.

Any compound of formula (I) referred to herein is intended to represent such specific compound as well as certain variations or forms. In particular, compounds referred to herein may have asymmetric centres and therefore exist in different enantiomeric or diastereomeric forms. Thus, any given compound of formula (I) referred to herein is intended to represent any one of a racemate, one or more enantiomeric forms, one or more diastereomeric forms, and mixtures thereof. Likewise, stereoisomerism or geometric isomerism about the double bond is also possible, therefore in some cases the molecule could exist as (*E*)-isomer or (*Z*)-isomer (*trans* and *cis* isomers). When the molecule contains several double bonds, each double bond will have its own stereoisomerism, that could be the same as, or different to, the stereoisomerism of the other double bonds of the molecule. Furthermore, compounds referred to herein may exist as atropisomers. All the stereoisomers including enantiomers, diastereoisomers, geometric isomers and atropisomers of the compounds referred herein, and mixtures thereof, are considered within the scope of the present invention.

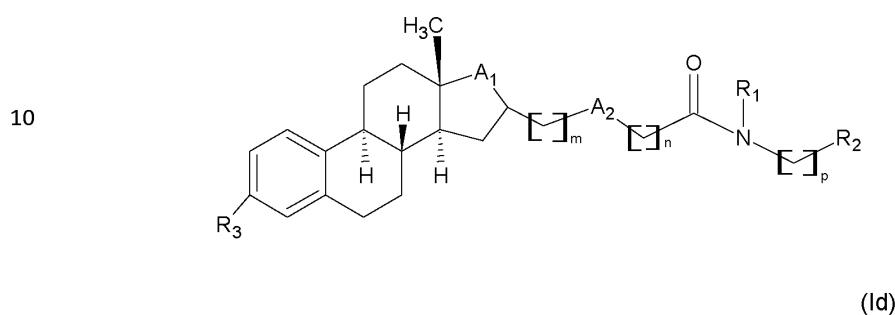
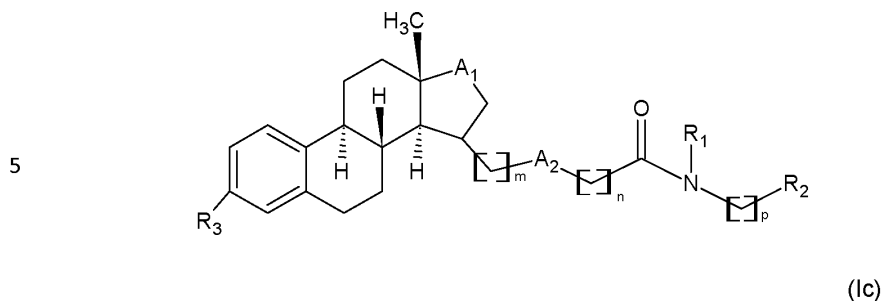
Furthermore, any compound of formula (I) referred to herein may exist as tautomer. Specifically, the term tautomer refers to one of two or more structural isomers of a compound that exist in equilibrium and are readily converted from one isomeric form to another.

In one embodiment of the invention, the compound of the invention is one of formula (Ia) or (Ib):



wherein R_1 to R_3 , A_1 , A_2 , m , n , and p are as defined in the first aspect of the invention.

In another embodiment the compound of the invention is of formula (Ic) or (Id):



wherein R_1 to R_3 , A_1 , A_2 , m , n , and p are as defined in the first aspect of the invention.

- 15 In one embodiment of the invention, the compound is one wherein A_1 is $C(O)$. In an alternative embodiment, A_1 is CR_{z1} , wherein R_{z1} is selected from OH , $=N-OH$, $=N-OCH_3$, NH_2 , F , OCH_3 , $OCOCH_3$, OSO_2NH_2 ; particularly R_{z1} is OH . In one embodiment, A_1 is selected from $C(O)$ or $CHOH$.

- 20 In one embodiment of the invention, the compound of the invention is one wherein m is other than 0, and $A_2 = CH_2$. In particular, m is 1, $A_2 = CH_2$ and n is 0.

In an alternative embodiment, the compound of the invention is one wherein $m = 0$, and $A_2 = -O-$. In another embodiment, the compound of the invention is one wherein $m = 0$, and $A_2 = -O-$ and n is other than 0, particularly $n = 1$.

- 25 In one embodiment of the invention, the compound of the invention is one wherein R_1 is hydrogen.

In one embodiment of the invention, the compound of the invention is one wherein R_3 is bromoethyl, particularly 2-bromoethyl.

In one embodiment of the invention, the compound of the invention is one wherein p is 0 when R₂ represents a 5-membered heterocyclic aromatic ring.

In another embodiment of the invention, the compound of the invention is one wherein p is 1 when R₂ represents a 6-membered heterocyclic aromatic ring.

- 5 In one embodiment of the invention, the compound of the invention is one wherein R₂ is a heterocyclic aromatic ring having 5 or 6 members. Particularly R₂ is a heterocyclic aromatic ring having one or two heteroatoms. In another particular embodiment, R₂ is a heterocyclic aromatic ring having one or two heteroatoms selected from N or S. In one embodiment R₂ is a thiazolyl ring wherein R_{Z2} is as defined above, particularly R_{Z2} is selected from H and
10 (C₁-C₅)alkyl. In an alternative embodiment R₂ represents a pyridinyl ring, wherein R_{Z2} is as defined above, particularly R_{Z2} is selected from H and (C₁-C₅)alkyl.

In a further embodiment, the compound of the invention is selected from the group consisting of:

- 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15β-yl]-N-(5-methyl-1,3-thiazol-2-
15 yl)propanamide (PBRM-II);
- 3-[3-(2-bromoethyl)-17β-hydroxyestra-1(10),2,4-trien-15β-yl]-N-(5-methyl-1,3-thiazol-2-yl)propanamide (PBRM-II-OH);
- 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-(5-methyl-1,3-thiazol-2-yl)propanamide (PBRM-III);
- 20 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (PBRM-IV);
- 3-[3-(2-bromoethyl)-17-β-hydroxyestra-1(10),2,4-trien-16β-yl]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (PBRM-V);
- 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-
25 yl)methyl]propanamide (PBRM-VII);
- 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]acetamide (PBRM-VIII);
- 3-[3-(2-bromoethyl)-17β-hydroxyestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]propanamide (PBRM-IX);
- 30 3-[3-(2-bromoethyl)-17β-hydroxyestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]acetamide (PBRM-X);

3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15 β -yl]-N-[(pyridin-3-yl)methyl]propanamide (PBRM-XI);

3-[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-15 β -yl]-N-[(pyridin-3-yl)methyl]propanamide (PBRM-XII);

- 5 2-[[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15 β -yl]oxy]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (PBRM-XIII); and

2-[[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-15 β -yl]oxy]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (PBRM-XIV);

and any salt or stereoisomer thereof.

- 10 In a third aspect, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of the compounds of the invention, as defined above.

By "therapeutically effective amount", it is understood the amount of the compound(s) that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disease which is addressed.

- 15 The precise therapeutic dose of the component(s), as well as the amount of the compound(s) of the invention, may depend on several variables. Some of these would be: route of administration, time of drug release (e.g., instant or extended), administration schedule, pain severity, condition of the patient, and the like.

- The pharmaceutical compositions can be prepared as a liquid, semi-solid or solid dosage
20 form, for example in the form of solutions for injection, drops, juices, syrups, sprays, suspensions, tablets, patches, capsules, dressings, suppositories, ointments, creams, lotions, gels, emulsions, aerosols or in multiparticulate form, for example in the form of pills or granules, if appropriate compressed into tablets, decanted into capsules or suspended in a liquid, or administered as such.

- 25 These compositions can be prepared with the aid of conventional means, devices, methods or processes known in the art.

- Pharmaceutically acceptable adjuvants, vehicles or excipients which may be used in such compositions are adjuvants, vehicles or excipients known to those skilled in the art or commonly used in the preparation of therapeutic compositions, which may be selected, for
30 example, from the group consisting of excipients, fillers, solvents, diluents, surfactants, colorants, preservatives, disintegrants, sliding agents, lubricants, flavoring agents or binders.

The term "pharmaceutically acceptable" refers to pharmaceutically acceptable materials, compositions or vehicles. Each component must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the pharmaceutical composition. It must also be suitable for use in contact with the tissue or organ of humans without
5 excessive toxicity, irritation, allergic response, immunogenicity or other problems or complications commensurate with a reasonable benefit/risk ratio in animals and, particularly, in humans.

The selection of physiologically compatible adjuvants or the number of adjuvants to be used depends on the form of administration of the pharmaceutical composition, i.e., oral,
10 subcutaneous, parenteral, intravenous, intraperitoneal, intradermal, intramuscular, intranasal, buccal, rectal, otic or intratympanic. Preparations in the form of tablets, dragees, capsules, granules, pills, drops, in particular otic drops, juices or syrups are preferably suitable for oral administration; solutions, suspensions, easily reconstitutable dry preparations or also sprays are preferably suitable for parenteral, topical or inhalation
15 administration. The compounds in accordance with the invention used in the pharmaceutical composition in accordance with the invention in a depot, in a dissolved form or in a dressing, or if appropriate having added other agents favoring penetration into the skin, are preparations suitable for percutaneous administration. The preparation forms administrable orally or percutaneously can also release the respective compound according
20 to the invention in a delayed form.

For instance, for oral administration in the form of a tablet or capsule, the active drug components can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulphate, mannitol, sorbitol and the like; for oral
25 administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and colouring agents can also be incorporated into the mixture. Suitable binders include starch, gelatine, natural sugars such as glucose or beta-lactose, corn sweeteners,
30 natural and synthetic gums such as acacia, tragacanth, or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum, and the like.

Gelatine capsules contain the active ingredient and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period
5 of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

Liquid dosage forms for oral administration can contain colouring and flavouring to increase patient acceptance.

10 The dosage administered of the pharmaceutical composition will, of course, vary depending on the use and known factors such as the age, health, and weight of the recipient; nature and extent of symptoms, concurrent treatments, if any, frequency of treatment, and the effect desired. The recipient may be any type of mammal, but is preferably a human.

The present invention also provides therapeutic and prophylactic methods and uses based
15 on the compounds and compositions provided by the present invention.

Throughout the present description, the term "treatment" includes, but is not limited to, alleviating, diminishing or eliminating one or more symptoms of the disorder; reducing the degree of the disease, stabilizing (i.e., not worsening) the condition, delaying or slowing the progression, alleviating or improving its condition, and remitting (whether total or partial).

20 As used in the present invention, the term "prevention" refers to preventing the onset of the pain from occurring in a patient who is predisposed, but who does not yet have symptoms of the disease.

"Estrogen-dependent condition" refers to a disease, a condition, a tumor which initiation, and/or proliferation and/or growth is stimulated by estrogens. "Estrogen-dependent
25 condition can be a benign condition or a malignant condition":

Conditions which are estrogen-dependent can either be benign (local proliferation without metastasis) or malignant (local proliferation associated with metastasis). A malignant condition is typically a cancer.

In one embodiment, the subject suffers from a malign estrogen-dependent disease or
30 disorder, such as cancer. Illustrative non-limitative examples of cancers are breast cancer, lung cancer, prostate cancer, endometrial cancer, uterine cancer, and ovarian cancer, among others.

In another embodiment the subject suffers from a benign estrogen-dependent disease or disorder such as endometriosis, uterine fibroids, uterine leiomyoma, adenomyosis, dysmenorrhea, menorrhagia, metrorrhagia, prostatic hyperplasia, urinary dysfunction, polycystic ovarian syndrome, lower urinary tract syndrome, multiple
5 sclerosis, obesity, rheumatoid arthritis, colon cancer, tissue wounds, skin wrinkles or cataracts.

In another embodiment of the compositions, methods and uses, the compounds of the invention are administered in combination with one or more other therapeutic agents suitable in the treatment of estrogen-dependent diseases.

10 The term "therapeutic agent" is used to describe an agent, other than a compound according to the present invention, which is used in combination with the present compounds as an agent with biological activity to assist in effecting an intended therapy, inhibition and/or prevention/prophylaxis for which the present compounds are used. Preferred bioactive agents for use herein include those agents which have pharmacological
15 activity similar to that for which the present compounds are used or administered and include for example, anti-cancer agents, antiviral agents, especially including anti-HIV agents and anti-HCV agents, antimicrobial agents, antifungal agents, non-steroidal anti-inflammatory compounds (NSAID), retinoid compounds, matrix metallo-protease inhibitors, an anti-estrogens, GnRH agonists or antagonists, selective progesterin receptor modulators
20 (SPRM), angiogenesis inhibitors, progesterin like compounds, aromatase inhibitors, 17 β -HSD-7 inhibitors, 17 β -HSD5 inhibitors, or any combination thereof.

A non-steroidal anti-inflammatory compound (NSAID), according to the invention, is e.g. a compound selected from acetyl salicylic acid, indometacin, sulindac, phenylbutazone, diclofenac, fentiazac, ketorolac) piroxicam, tenoxicam, mecloxicam, meloxicam,
25 cinnoxicam, ibufenac, ibuprofen, naproxen, ketoprofen, nabumetone, niflumic acid and nimesulide, or a pharmaceutically acceptable salt thereof. Preferred NSAIDs are diclofenac, piroxicam, tenoxicam, mecloxicam, meloxicam, ibufenac, ibuprofen, naproxen and ketoprofen, or a pharmaceutically acceptable salt thereof.

Examples of retinoid compounds according to the invention include, for example, Accutane;
30 Adapalene; Allergan AGN-193174; Allergan AGN-193676; Allergan AGN-193836; Allergan AGN-193109; Aronex AR-623; BMS-181162; Galderma CD-437; Eisai ER-34617; Etrinate; Fenretinide; Ligand LGD-1550; Ixcalcitrol; Maxia Pharmaceuticals MX-781; mofarotene; Molecular Design MDI-101; Molecular Design MDI-301; Molecular Design MDI-403; Motretinide; Eisai 4-(2-[5-(4-methyl-7-ethylbenzofuran-2-yl)pyrrolyl])benzoic acid; Johnson
35 & Johnson N-[4-[2-thyl-1-(1H-imidazol-1-yl)butyl]phenyl]-2-benzothiazolamine; Soriatane;

Roche SR-11262; Tocoretinate; Advanced Polymer Systems trans-retinoic acid; UAB Research Foundation UAB-8; Tazorac; TopiCare; Taiho TAC-101; and Vesanoid.

Examples of matrix metallo-protease inhibitors according to the invention include known:

- 1-cyclopropyl-N-hydroxy-4-[[4-[4-(trifluoromethoxy)phenoxy]phenyl]sulfonyl]-4-piperidinecarboxamide monohydrochloride;
- 5 N-hydroxy-1-(phenylmethyl)-4-[[4-[4-(trifluoromethoxy)phenoxy]-1-piperidinyl]sulfonyl]-4-piperidinecarboxamide monohydrochloride;
- N-hydroxy-1-(pyridinylmethyl)-4-[[4-[4-(trifluoromethyl)phenoxy]phenyl]sulfonyl]-4-piperidinecarboxamide dihydrochloride;
- 10 N-hydroxy-2,3-dimethoxy-6-[[4-[4-(trifluoromethyl)phenoxy]-1-piperidinyl]sulfonyl]-benzamide;
- N-hydroxy-1-(4-pyridinylmethyl)-4-[[4-[4-(trifluoromethyl)phenoxy]phenyl] sulfonyl]-4-piperidinecarboxamide dihydrochloride;
- N-hydroxy-1-(3-pyridinylmethyl)-4-[[4-[4-(trifluoromethyl)phenoxy]phenyl] sulfonyl]-4-piperidinecarboxamide dihydrochloride;
- 15 N-hydroxy-1-(2-pyridinylmethyl)-4-[[4-[4-(trifluoromethyl)phenoxy]phenyl]sulfonyl]-4-piperidinecarboxamide monohydrochloride;
- British Biotech BB-2516 (marimastat), N4-[2,2-dimethyl-1-[(methylamino)carbonyl]-propyl]-N1,2-dihydroxy-3-(2-methylpropyl)-, [2S-[N4(R*), 2R*, 3S*]]-);
- 20 BMS 275291 disclosed in WO 97/19075;
- Bayer Ag Bay-12-9566 (tanomastat), 4-[(4'-chloro[1,1-diphenyl]-4-yl)oxyl-2-[(phenylthio)methyl]butanoic acid;
- Agouron Pharmaceuticals AG-3340, N-hydroxy-2,2'-dimethyl-4-[[4-(4-pyridinyloxy)phenyl]sulfonyl]-3-thiomorpholinecarboxamide;
- 25 CollaGenex Pharmaceuticals CMT-3 (metastat), 6-demethyl-6-deoxy-4-dedimethylaminotetracycline, batimastat (BB-94); and
- Chiroscience D-2163, 2-[1S-([(2R,S)-acetylmercapto-S-phthalimido]pentanoyl-L-leucyl)amino-3-methylbutyl]imidazole.

An anti-estrogen, e.g. a selective estrogen receptor modulator (SERM), is preferably a SERM devoid of uterotrophic activity. Examples of SERMs, according to the invention, are tamoxifen, toremifene, arzoxifene, idoxifene, EM 800, fulvestrant and droloxifene.

Examples of GnRH (LHRH) agonists according to the invention are, e.g., leuporelin, deslorelin, triptorelin, buserelin, nafarelin, goserelin, avorelin, histerelin, compound PTL 03001 (5-oxo-L-propyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-tryptophyl-L-leucyl-L-arginyll-N-ethyl-L-prolinamide) (Peptech), compound AN 207 (6-[N6-[5-[2-[1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-4-[[2,3,6-trideoxy-3-(2,3-dihydro-1H-pyrrol-1-yl).alpha.-L-lyxo-hexopyranosyl]oxy]-2-naphthaceny]-1,5-dioxopentyl]-D-lysine]-, (2S-cis)-) (ASTA Medica Inc.), compound AN 238 L-threoninamide, N-[5-[2-[(2S,4S)-1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-4-[[2,3,6-trideoxy-3-(2,3-dihydro-1H-pyrrol-1-yl).alpha.-L-lyxo-hexopyranosyl]oxy]-2-naphthaceny]-2-oxoethoxy]-1,5-dioxopentyl]-D-phenylalanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-valyl-L-cysteinylcyclic (2.fwdarw.7)-disulfide (ASTA Medica Inc.) and compound SPD 424 (LHRH hydrogel implant) (Shire Pharmaceuticals Group), or a pharmaceutically acceptable salt thereof.

Preferred examples are triptorelin, leuporelin and goserelin, or a pharmaceutically acceptable salt thereof, in particular triptorelin or a pharmaceutically acceptable salt thereof, e.g. as triptorelin pamoate.

Examples of GnRH (LHRH) antagonists, according to the invention, are e.g. cetorelix, abarelix, ramorelix, teverelix, ganirelix, compounds A 75998 (Acetyl-D-(2-naphthyl)alanyl-D-(4-chlorophenyl)alanyl-D-(3-pyridyl)alanyl-seryl-(N-methyl)tyrosyl-N6-(nicotinoyl)-D-lysyl-leucyl-N6-(isopropyl)lysyl-propyl-D-alaninamide) and A 84861 (Tetrahydrofuran-2-(S)-ylcarbonyl-glycyl-D-(2-naphthyl)alanyl-D-(4-chloro)phenylalanyl-D-(3-pyridyl)-alanyl-L-(N-methyl)tyrosyl-D-[N6-(3-pyridylcarbonyl)]lysyl-L-leucyl-L-(N6-isopropyl)lysyl-L-propyl-D-alanyl-L-alaninamide) (Abbot Labs.), GnRH immunogen (Aphtron Co.), compound T 98475 (Isopropyl 3-(N-benzyl-N-methylaminomethyl)-7-(2,6-difluorobenzyl)-4,7-dihydro-2-(4-isobutyrylamino)phenyl)-4-oxothieno[2,3-bpyridine-5-carboxylate hydrochloride) (Takeda), and compound MI 1544 (Acetyl-D-tryptophyl-D-cyclopropyl-alanyl-D-tryptophyl-L-seryl-L-tyrosyl-D-lysyl-L-leucyl-L-arginyll-L-propyl-D-alaninamide), or a pharmaceutically acceptable salt thereof.

Examples of selective progesterin receptor modulators (SPRMs), according to the invention, are e.g. dienogest or a pharmaceutically acceptable salt thereof.

An angiogenesis inhibitor is e.g. an $\alpha\text{v}\beta 3$ integrin inhibitor, a protein kinase inhibitor, angiostatin, platelet factor 4 (endostatin), a VEGF inhibitor or thalidomide.

Vascular endothelial growth factor (VEGF) inhibitors and telomerase inhibitors are well known in the art.

Moreover known VEGF inhibitors or antagonists are agents which suppress angiogenesis by reducing binding of VEGF to cellular receptors, including but not limited to, for example
 5 blocking monoclonal antibodies against the growth factor (e.g. rhuMAbVEGF, Ryan et al., Toxicol Pathol 1999, 27:78-86), against the receptor (e.g. DC101 and derivatives, Witte et al., Cancer Metastasis Rev 1998, 17:155-61), soluble forms of VEGF receptors (e.g. soluble Flt, Aiello et al., Proc Natl Acad Sci U S A 1995, 92:10457-61), or compounds which directly antagonise interactions between VEGF and cell surface receptors (e.g. Fairbrother et al.,
 10 Biochemistry 1998,37:17754-64).

A protein kinase inhibitor, according to the invention, is for instance a tyrosine kinase inhibitor, in particular compounds 3-[4-(2-carboxyethyl-3,5-dimethylpyrrol-2-yl)methylidenyl]-2-indolinone, and 3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-2-indolinone.

Examples of $\alpha v \beta 3$ integrin inhibitors are known:

- 15 Vitaxin antibody (Ixsys); Merck KgaA EMD-121974, cyclo[RGDF-N(Me)V-];
 (10S)-10,11-dihydro-3-[3-(2-pyridinylamino)propoxy]-5H-dibenzo[a,d]cycloheptene-10-acetic acid;
 (2S)-7-[[[(1H-benzimidazol-2-ylmethyl)methylamino]carbonyl]-2,3,4,5-tetrahydro-4-methyl-3-oxo-1H-1,4-benzodiazepine-2-acetic acid;
 20 (2S)-2,3,4,5-tetrahydro-4-methyl-7-[[[(5-methyl-1H-imidazo[4,5-b]pyridin-2-yl)methyl]amino]carbonyl]-3-oxo-1H-1,4-benzodiazepine-2-acetic acid;
 (bR)-b-[[[(3R)-2-oxo-3-[2-(5,6,7,8-tetrahydro-[1,8]-naphthyridin-2-yl)ethyl]1-1-pyrrolidinyl]acetyl]amino]-d-(1H-indol-3-yl)pentanoic acid; and
 (3R)-N-[3-hydroxy-5-[(1,4,5,6-tetrahydro-5-hydroxy-2-pyrimidinyl)amino]benzoyl]-
 25 glycyl-3-(3-bromo-5-chloro-2-hydroxyphenyl)- β -alanine.

Angiostatin, endostatin and thalidomide are well known in the art. Pharmaceutically acceptable salts of the compound mentioned herein are well known in the art.

In one embodiment, the one or more other therapeutic agents are administered simultaneously, consecutively, or separately to the compound or composition of the
 30 invention.

To those skilled in the art, other objects, advantages or features of the invention will be apparent in part from the description or in part from the practice of the invention. The following examples are provided by way of illustration or are not intended to be limiting of the present invention.

5 EXAMPLES

Chemical synthesis of the compounds of the invention

I. Materials and Methods

Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). 2-(3-Bromopropoxy)-*tert*-butyldimethylsilane was purchased from Combiblock
10 (San Diego, CA, USA).

The usual solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and were used as received. Anhydrous dichloromethane (DCM), tetrahydrofuran (THF) and dimethylformamide (DMF) were obtained from Sigma-Aldrich.

Thin-layer chromatography (TLC) and flash-column chromatography were performed on
15 0.20-mm silica gel 60 F254 plates and with 230-400 mesh ASTM silica gel 60, respectively (E. Merck; Darmstadt, Germany).

Infrared (IR) spectra were recorded on a Horizon MB 3000 ABB FTIR spectrometer (Québec, QC, Canada), and only the significant bands are reported (in cm^{-1}).

Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz and 400 MHz for
20 ^1H and 75 MHz and 100.6 MHz for ^{13}C on a Bruker Avance NEO 300 and 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), acetone (2.05 and 28.9 ppm), methanol (3.31 and 49.0 ppm) or dimethylsulfoxide (2.49 and 39.5 ppm) for ^1H and ^{13}C NMR, respectively.

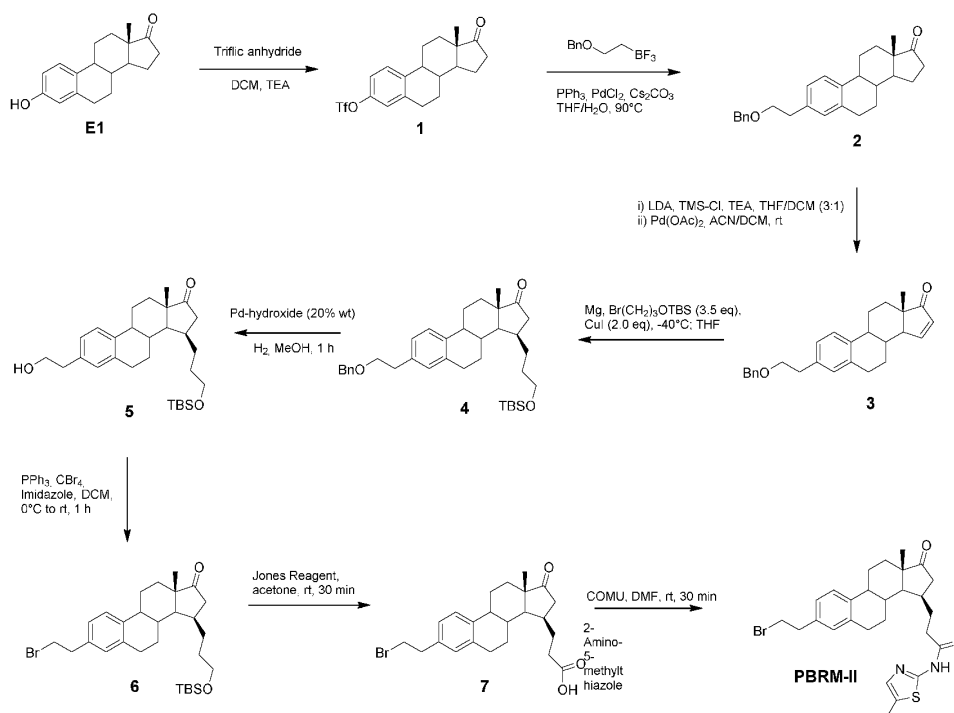
25 High-performance liquid chromatography (HPLC) analyses for chemical purities were performed on a Shimadzu Prominence instrument (Kyoto, Japan) using a diode array detector and an Altima C18 analytical reverse phase column (5 μm , 4.6 X 250 mm) applying the conditions stated (wavelength detection and solvent gradient).

Low-resolution mass spectra (LRMS) were recorded on a Shimadzu Prominence
30 instrument (Kyoto, Japan) equipped with a Shimadzu LCMS-2020 mass spectrometer and an APCI (atmospheric pressure chemical ionization) probe and expressed in m/z . The

Plateforme de diffraction des rayons X (Department of Chemistry, Université de Montréal, Montréal, QC, Canada) performed the X-ray analysis.

II. Synthesis of the compound of the invention 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15 β -yl]-N-(5-methyl-1,3-thiazol-2-yl)propanamide (PBRM-II)

5



Scheme 3. Synthesis of PBRM-II. Reagents and conditions.

17-oxoestra-1(10),2,4-trien-3-yl trifluoromethanesulfonate (1)

- 10 This compound was prepared following a literature procedure (*R. Maltais et al. Org. Process Res. Dev.* 2019, 23, 11, 2323-2334). ¹H NMR data was in full agreement with that reported in the literature.

3-[2-(benzyloxy)ethyl]estra-1(10),2,4-trien-17-one (2)

- This compound was prepared following a literature procedure (*R. Maltais et al. Org. Process Res. Dev.* 2019, 23, 11, 2323-2334). ¹H NMR data was in full agreement with that reported in the literature.
- 15

3-[2-(benzyloxy)ethyl]estra-1(10),2,4,15-tetraen-17-one (3)

To a solution of compound **2** (2.6 g, 6.7 mmol) in anhydrous THF (130 mL) was dropwise added lithium diisopropylamide (LDA) 1.5 M in THF (8.92 mL, 13.4 mmol) at -78°C under an argon atmosphere. The solution was stirred for 40 min and trimethylamine (TEA) (2.74 mL, 20.1 mmol) and trimethylsilyl chloride (TMSCl) (2.54 mL, 20.0 mmol) were sequentially added. The solution was then warmed to room temperature and stirred for an additional 30 min. The resulting solution was poured into a 10% sodium bicarbonate solution and extracted with DCM. The organic layer was dried over sodium sulfate and evaporated under reduce pressure. The crude compound was diluted in a mixture of ACN/DCM (64 mL/16 mL), Pd(OAc)₂ (1.5 g, 6.7 mmol) was added and the solution was stirred for 1 h at 40°C. The resulting solution was evaporated and the crude compound was purified by flash chromatography using EtOAc/hexanes (2:8) to give 1.4 g (54%) of compound **3**.

¹H NMR (CDCl₃): 1.11 (s, 3H), 1.47-2.54 (m, 8H), 2.89 (t, 2H, *J* = 7.0 Hz), 2.95 (m, 2H), 3.69 (t, 2H, *J* = 7.1 Hz), 4.54 (s, 2H), 6.09 (d, 1H, *J* = 5.4 Hz), 6.99 (s, 1H), 7.04 (d, 1H, *J* = 7.9 Hz), 7.22 (d, 1H, *J* = 7.9 Hz), 7.33 (m, 5H), 7.63 (d, 1H, *J* = 5.7 Hz). ¹³C NMR (Acetone-d₆): 20.3, 25.2, 26.5, 31.7, 35.5, 35.6, 45.3, 51.0, 55.9, 71.2, 72.2, 124.8, 126.4, 127.4 (3C), 128.2 (2C), 129.4, 131.3, 136.0, 136.6, 137.5, 139.1, 158.1, 211.2; LRMS for C₂₇H₃₄O₂ [M + H]⁺ 387.2 m/z.

3-[2-(benzyloxy)ethyl]-15β-(3-[[*tert*-butyl(dimethyl)silyl]oxy]propyl)estra-1(10),2,4-trien-17-one (4)

Magnesium powder (0.1-0.3 mm) (282 mg, 11.8 mmol) and a crystal of iodide was deposited in a 100 mL round bottom flask and flame dried under an argon atmosphere and followed by dropwise addition (over 20 min) of a solution of 2-(3-bromopropoxy)-*tert*-butyldimethylsilane (2.29 g, 9.04 mmol) in anhydrous THF (40 mL) at room temperature. The solution was then stirred for 90 min at room temperature. The resulting trouble gray solution was left to stand 5 min until a solid black depot accumulated at the bottom of the flask. The solution was then transferred via a cannula to a 200 mL round flame dried bottom flask and without transferring the black solid depot. The solution was cooled at -40°C and CuI (984 mg, 5.18 mmol) was then rapidly added in one shot. The solution was vigorously stirred for 15 min at this temperature and the color of the solution changed gradually from pale gray to pale gray purple color. A solution of compound **3** (1.0 g, 2.59 mmol) in anhydrous THF (40 mL) was then dropwise added over 70 min at -40°C (1 drop per 4-5 seconds) and then stirred at -40°C for an additional 15 min. Glacial acetic acid (1.0 mL) was then dropwise added at -40°C and stirred for 30 min at this temperature. The resulting solution was poured into a 10% ammonium chloride solution and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated

under reduced pressure to give 2.3 g of crude material. The crude compound was purified by flash chromatography with EtOAc/hexanes (5:95 to 10:90) to give 1.02 g (70%) of compound 4.

¹H NMR (Acetone-d₆): 0.09 (s, 6H), 0.93 (s, 9H), 1.05 (s, 3H), 1.42-2.38 (m, 15H), 2.83-2.93 (m, 5H), 3.66-3.74 (m, 4H), 4.53 (s, 2H), 6.99 (s, 1H), 7.03 (d, 1H, *J* = 8.0 Hz), 7.21 (d, 1H, *J* = 8.0 Hz), 7.23-7.35 (m, 5H). ¹³C NMR (Acetone-d₆): -6.0 (2C), 17.2, 17.9, 25.3, 25.4 (3C), 26.6, 27.0, 29.1, 32.6, 33.9, 34.1, 35.6, 36.0, 42.0, 44.9, 46.7, 52.7, 62.2, 71.2, 72.3, 124.9, 125.1, 126.3, 127.2, 127.4 (2C), 128.1 (2C), 129.4, 136.1, 136.5, 137.8, 139.1, ~220.0; LRMS for C₃₆H₅₅O₃Si [M + H]⁺ 562.4 m/z.

10 15β-(3-[[*tert*-butyl(dimethyl)silyl]oxy]propyl)-3-(2-hydroxyethyl)estra-1(10),2,4-trien-17-one (5)

To compound 4 (6.0 g, 10.7 mmol) in a mixture of MeOH/DCM (270 mL/30 mL) at room temperature and under an atmosphere of argon was added palladium hydroxide (20% wt) on charcoal (600 mg). The round bottom flask was purged three times with hydrogen and stirred at room temperature for 1 h under an atmosphere of hydrogen. The resulting solution was filtered over celite, washed with MeOH and evaporated under reduce pressure to give 5.0 g of crude compound 5. The compound was evaporated two times with DCM to remove residual trace of MeOH. The crude compound was purified by flash chromatography with EtOAc/hexanes (4:6) to give 3.5 g (70%) of compound 5.

20 ¹H NMR (Acetone-d₆): 0.08 (s, 6H), 0.92 (s, 9H), 1.05 (s, 3H), 1.43-2.43 (m, 16H), 2.73-2.94 (m, 5H), 3.66-3.77 (m, 4H), 6.97 (s, 1H), 7.01 (d, 1H, *J* = 8.0 Hz), 7.21 (d, 1H, *J* = 8.0 Hz). ¹³C NMR (Acetone-d₆): -6.0 (2C), 17.2, 17.9, 25.3, 25.4 (3C), 26.7, 27.0, 29.1, 32.6, 33.9, 34.1, 36.0, 39.0, 42.0, 44.9, 46.7, 52.7, 62.3, 63.1, 124.8, 126.3, 129.4, 136.1, 136.8, 137.8, ~220.0; LRMS for C₂₉H₄₇O₃Si [M + H]⁺ 471.3 m/z.

25 3-(2-bromoethyl)-15β-(3-[[*tert*-butyl(dimethyl)silyl]oxy]propyl)estra-1(10),2,4-trien-17-one (6)

30 To a solution of crude compound 5 (3.45 g, 7.32 mmol) in DCM (300 mL) at 0°C was added imidazole (4.98 g, 73.1 mmol) and triphenylphosphine (7.8 g, 29.7 mmol). To the later solution was slowly added (over 2 min) at 0°C a solution of carbon tetrabromide (9.8 g, 29.7 mmol) diluted in DCM (30 mL). The solution was stirred at 0 °C for 5 min and then allowed to return to room temperature and stirred for 45 min. The resulting solution was poured into sodium bicarbonate solution (10%), extracted with DCM, filtered over phase separator syringe and evaporated under reduced pressure. Purification by flash chromatography using EtOAc/hexanes (1:9) gave 2.93 g (75%) of brominated compound 6.

¹H NMR (Acetone-d₆): 0.08 (s, 6H), 0.93 (s, 9H), 1.05 (s, 3H), 1.44-2.44 (m, 16H), 2.79-2.96 (m, 2H), 3.11 (t, 2H, *J* = 7.5 Hz), 3.66 (t, 2H, *J* = 7.5 Hz), 3.64-3.74 (m, 2H), 7.03 (s, 1H), 7.06 (d, 1H, *J* = 8.0 Hz), 7.26 (d, 1H, *J* = 8.0 Hz). ¹³C NMR (Acetone-d₆): -6.0 (2C), 17.2, 17.9, 25.2, 25.4 (3C), 26.6, 27.0, 29.1, 32.6, 33.3, 33.9, 34.1, 35.9, 38.7, 42.0, 44.9, 46.7, 52.7, 62.3, 125.1, 126.0, 129.1, 136.4, 136.5, 138.6, ~220.0; LRMS for C₂₉H₄₆BrO₂Si [M + H]⁺ 533.2 and 535.1 m/z.

3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15β-yl]propanoic acid (7)

To a solution of compound **6** (2.93 g, 5.49 mmol) in acetone (300 mL) at 0°C was added Jones reagents (3.0 mL) and the reaction mixture was stirred 30 min at this temperature. The resulting solution was quenched with isopropanol (5 mL), stirred 15 min, and then poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure to give 2.4 g of crude compound which was used as such for the next step.

¹H NMR (Acetone-d₆): 1.07 (s, 3H), 1.39-2.46 (m, 17H), 2.92 (m, 2H), 3.11 (t, 2H, *J* = 7.5 Hz), 3.66 (t, 2H, *J* = 7.5 Hz), 7.03 (s, 1H), 7.06 (d, 1H, *J* = 8.0 Hz), 7.26 (d, 1H, *J* = 7.9 Hz), 10.6 (broad s, 1H). ¹³C NMR (Acetone-d₆): 17.1, 25.2, 25.8, 26.5, 29.0, 32.8, 33.4, 33.7, 34.1, 35.8, 38.7, 41.4, 45.0, 46.6, 52.6, 125.1, 126.0, 129.1, 136.4, 136.6, 138.6, 173.7, ~220.0; LRMS for C₂₃H₃₀BrO₃ [M + H]⁺ 433.1 and 435.1 m/z.

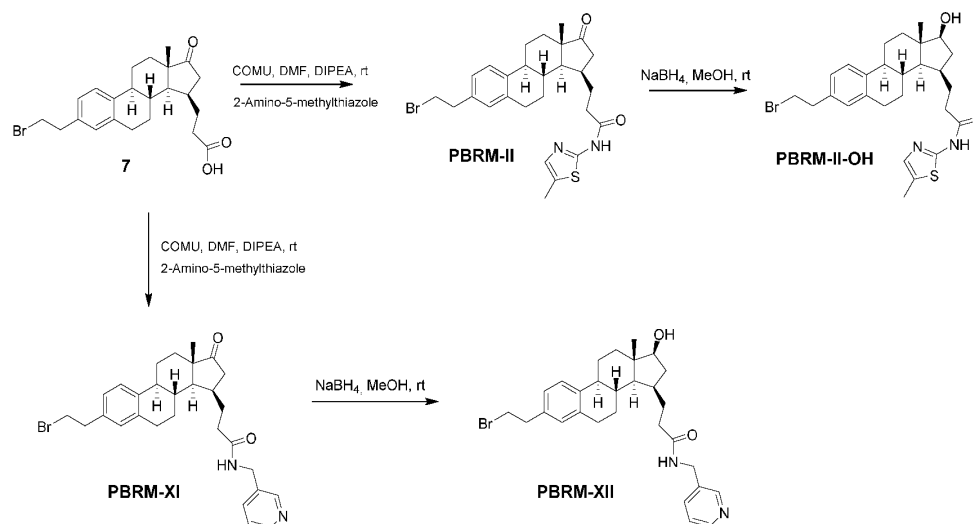
3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15β-yl]-N-(5-methyl-1,3-thiazol-2-yl)propanamide (PBRM-II)

To a solution of compound **7** (900 mg, 2.08 mmol) in anhydrous DMF (5 mL) was added (1-cyano-2-ethoxy-2-oxoethylidenaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (978 mg, 2.28 mmol) and the mixture was stirred 5 min before the addition of 2-amino-5-methylthiazole (474 mg, 4.15 mmol) and diisopropylethylamine (DIPEA) (723 μL, 4.6 mmol). The resulting solution was stirred at room temperature for 30 min. The solution was then poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude compound was purified by flash chromatography using EtOAc/Hexanes (1:1 to 7:3) to give 835 mg (76%) of PBRM-II.

¹H NMR (CDCl₃): 1.06 (s, 3H), 1.42-2.65 (m, 16H), 2.42 (s, 3H), 2.93 (m, 2H), 3.11 (t, 2H, *J* = 7.7 Hz), 3.56 (t, 2H, *J* = 7.7 Hz), 6.96 (s, 1H), 7.00 (d, 1H, *J* = 8.0 Hz), 7.05 (d, 1H, *J* = 1.2 Hz), 7.24 (d, 1H, *J* = 8.3 Hz), 11.0 (s, 1H); ¹³C NMR (CDCl₃): 11.6, 17.8, 25.3, 26.1, 26.7, 29.2, 33.0, 33.8, 33.9, 35.5, 35.7, 39.0, 42.0, 45.0, 47.1, 52.8, 125.3, 126.0, 127.8, 129.2, 133.0, 136.5, 136.7, 138.5, 158.2, 170.1, 220.2; LRMS for C₂₇H₃₄BrN₂O₂S [M + H]⁺ 529.2 and 531.2 m/z. HPLC purity = 98.7%.

III. Synthesis of other compounds of the invention

Chemical synthesis of PBRM-II-OH, PBRM-XI and PBRM-XII



Scheme 4. Synthesis of PBRM-II-OH, PBRM-XI and PBRM-XII. Reagents and conditions

5

3-[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-15 β -yl]-N-(5-methyl-1,3-thiazol-2-yl)propanamide (PBRM-II-OH)

To a solution of **PBRM-II** (50 mg, 0.094 mmol) in MeOH (3 mL) was added sodium borohydride (11 mg, 0.028 mmol). The resulting solution was stirred at 0°C for 1 h before
 10 to be poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude compound was purified by flash chromatography using EtOAc/hexanes (8:2 to 10:0) to give 40 mg (80%) of **PBRM-II-OH**.

¹H NMR (CDCl₃): 0.90 (s, 3H), 1.20-2.62 (m, 17H), 2.85 (m, 2H), 3.10 (t, 2H, *J* = 7.7 Hz),
 15 3.55 (t, 2H, *J* = 7.7 Hz), 3.73 (t, 1H, *J* = 8.6 Hz), 6.93 (s, 1H), 6.98 (d, 1H, *J* = 8.0 Hz), 7.05 (d, 1H, *J* = 0.8 Hz), 7.23 (d, 1H, *J* = 8.0 Hz), 12.1 (s, 1H); ¹³C NMR (CDCl₃): 11.6, 14.6, 25.4, 27.6, 27.7, 29.3, 33.0, 35.0, 35.1, 35.7, 38.0, 38.6, 39.0, 43.0, 45.0, 52.0, 81.7, 125.2, 125.9, 127.4, 129.1, 133.0, 136.3, 137.1, 139.2, 158.3, 170.8; LRMS for C₂₇H₃₆BrN₂O₂S [M + H]⁺ 531.0 and 533.0 m/z. HPLC purity = 99.4%.

20

3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15 β -yl]-N-[(pyridin-3-yl)methyl]propanamide (PBRM-XI)

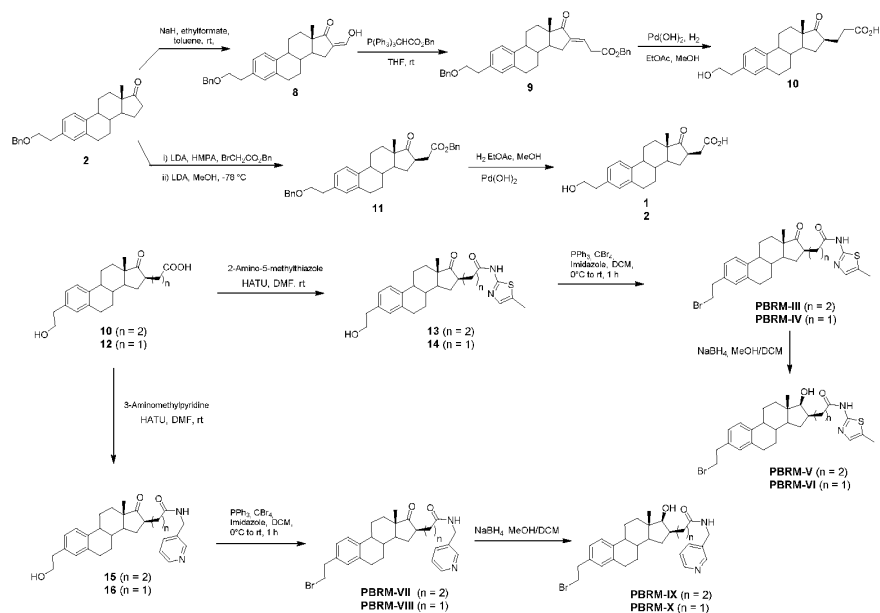
To a solution of compound **7** (50 mg, 0.094 mmol) in anhydrous DMF (2 mL) was added (1-cyano-2-ethoxy-2-oxoethylidenaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (45 mg, 0.105 mmol) and the mixture was stirred 5 min before the addition of 1-(pyridin-3-yl)methanamine (474 mg, 4.15 mmol) and diisopropylethylamine (DIPEA) (55 μ L, 0.32 mmol). The resulting solution was stirred at room temperature for 30 min. The solution was then poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude compound was purified by flash chromatography using DCM/MeOH (95:5 to 9:1) to give 55 mg (95%) of **PBRM-XI**.

^1H NMR (Acetone- d_6): 1.04 (s, 3H), 1.30-2.48 (m, 18H), 2.89 (m, 2H), 3.11 (t, 2H, $J = 7.5$ Hz), 3.66 (t, 2H, $J = 7.5$ Hz), 4.44 (d, 2H, $J = 6.0$ Hz), 7.02 (s, 1H), 7.05 (d, 1H, $J = 8.0$ Hz), 7.25 (d, 1H, $J = 8.0$ Hz), 7.30 (m, 1H), 7.72 (d, 2H, $J = 7.8$ Hz), 8.46 (d, 1H, $J = 4.0$ Hz), 8.55 (s, 1H); ^{13}C NMR (Acetone- d_6): 17.2, 25.2, 26.4, 26.6, 29.0, 33.4, 33.8, 34.1, 35.2, 35.8, 38.7, 40.3, 41.6, 44.9, 46.7, 52.7, 123.3, 125.1, 126.0, 129.2, 135.0 (2C), 136.4, 136.6, 138.6, 148.3, 149.2, 172.1, ~220.0; LRMS for $\text{C}_{29}\text{H}_{36}\text{BrN}_2\text{O}_2$ $[\text{M} + \text{H}]^+$ 523.2 and 525.2 m/z. HPLC purity = 99.2%.

3-[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-15 β -yl]-N-[(pyridin-3-yl)methyl]propanamide (PBRM-XII)

To a solution of **PBRM-XI** (20 mg, 0.032 mmol) in MeOH (1 mL) was added sodium borohydride (4 mg, 0.105 mmol). The resulting solution was stirred at 0°C for 1 h before to be poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude compound was purified by flash chromatography using DCM/MeOH (94:6) to give 8 mg (40%) of **PBRM-XII**.

^1H NMR (MeOH- d_4): 0.88 (s, 3H), 1.23-2.33 (m, 18H), 2.83 (m, 2H), 3.06 (t, 2H, $J = 7.5$ Hz), 3.56 (t, 2H, $J = 7.5$ Hz), 3.64 (t, 1H, $J = 8.6$ Hz), 4.40 (s, 1H), 4.60 (br s, 1H), 6.93 (s, 1H), 6.96 (d, 1H, $J = 8.0$ Hz), 7.20 (d, 1H, $J = 7.9$ Hz), 7.41 (dd, 1H, $J_1 = 4.9$ Hz, $J_2 = 7.8$ Hz), 7.79 (d, 1H, $J = 7.9$ Hz), 8.43 (br s, 1H), 8.49 (br s, 1H); ^{13}C NMR (MeOH- d_4): 15.3, 26.7, 28.8, 30.0, 30.3, 33.9, 36.1 (2C), 37.2, 38.5, 40.0, 40.1, 41.6, 44.1, 46.3, 53.2, 82.5, 125.3, 126.1, 126.9, 130.1, 136.9, 137.6, 137.7, 138.0, 140.4, 148.8, 149.5, 176.2; LRMS for $\text{C}_{29}\text{H}_{38}\text{BrN}_2\text{O}_2$ $[\text{M} + \text{H}]^+$ 525.1 and 527.1 m/z. HPLC purity = 98.2%.

Chemical synthesis of PBRM-III to PBRM-X

Scheme 5. Synthesis of PBRM-III to PBRM-X from intermediate compound **2**. Reagents and conditions. PBRM-VI was not obtained from PBRM-IV.

3-[2-(benzyloxy)ethyl]-16-(hydroxymethylene)-estra-1(10),2,4-trien-17-one (8)

To an ice cooled solution of compound **2** (500 mg, 1.29 mmol) in toluene (7 mL) was added NaH (60% mineral oil, 731 mg, 12.9 mmol). Ethyl formate (1 mL, 12.9 mmol) was added to the suspension and stirring was continued overnight at room temperature. The solution was quenched with a 10% HCl solution and extracted with EtOAc. The organic phase was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The oily residue was purified by flash chromatography using EtOAc/hexanes (1:4) to give 424 mg (79%) of compound **8** as a syrup.

^1H NMR (DMSO- d_6): 0.79 (s, 3H), 1.33-2.60 (m, 11H), 2.73-2.80 (m, 4H), 3.58 (t, 2H, J = 6.9 Hz), 4.45 (s, 2H), 6.91 (s, 1H), 6.96 (d, 1H, J = 8.0 Hz), 7.16 (d, 1H, J = 8.0 Hz), 7.25-7.34 (m, 5H), 7.38 (s, 1H), 10.59 (bs, 1H); ^{13}C NMR (DMSO- d_6): 14.3, 24.0, 25.4, 26.2, 28.8, 31.4, 35.0, 37.2, 43.8, 47.7, 48.4, 70.6, 71.7, 113.2, 124.9, 126.1, 127.2, 127.3, 127.4, 128.2 (2C), 129.1, 135.9, 136.0, 137.4, 138.5, 150.1, 208.6.

(E)-benzyl 3-[3-(2-(benzyloxy)ethyl)-17-oxoestra-1(10),2,4-trien-16-ylidene]propanoate (9)

To a solution of compound **8** (540 mg, 1.3 mmol) in THF (5 mL) was added benzyl(triphenylphosphoranylidene)acetate (640 mg, 1.56 mmol). The mixture was stirred at room temperature overnight. After evaporation, the crude material was purified by flash chromatography using EtOAc/hexanes (1:9) to give benzyl ester **9** (570 mg, 80%) as a syrup and a mixture of E (83%) and Z (17%) isomers.

¹H NMR (CDCl₃): 0.96 (s, 3H), 1.40-2.55 (m, 11H), 2.62-2.75 (m, 1H), 2.92-2.97 (m, 4H), 3.32 (d, 2H, *J* = 7.3 Hz), 3.75 (t, 2H, *J* = 7.2 Hz), 4.59 (s, 2H), 5.22 (s, 2H), 6.84 (t, 1H, *J* = 7.4 Hz), 7.04 (s, 1H), 7.09 (d, 1H, *J* = 8.0 Hz), 7.28 (d, 1H, *J* = 8.0 Hz), 7.34-7.42 (m, 10H); ¹³C NMR (CDCl₃): 14.2, 25.6, 26.3, 26.6, 29.2, 31.4, 35.0, 35.7, 37.6, 44.1, 47.6, 48.3, 66.7, 71.1, 72.8, 125.2, 126.3, 127.2, 127.3, 127.4, 127.5, 128.0, 128.1, 128.2, 128.3, 128.4, 129.4, 135.4, 136.1, 136.2, 136.3, 137.1, 137.4, 138.3, 139.9, 169.7, 207.6; LRMS for C₃₇H₄₀O₄ [M + H]⁺ 549.3 m/z.

3-[3-(2-hydroxyethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]propanoic acid (10)

To a solution of compound **9** (300 mg, 0.53 mmol) in 1:1 EtOAc/MeOH mixture (10 mL) under an argon atmosphere was added palladium hydroxide on carbon (loading 20% weight, 60 mg). The flask was purged three times with hydrogen and stirred at room temperature for 1 h. The suspension was filtered over celite, washed with MeOH and evaporated under reduce pressure. The crude compound was purified by flash chromatography using MeOH/DCM (1:99 to 7:93) to give 161 mg (82%) of acid **10**.

¹H NMR (CDCl₃): 0.87 (s, 3H), 1.37-2.48 (m, 14H), 2.51 (t, 2H, *J* = 7.2 Hz), 2.81 (t, 2H, *J* = 6.6 Hz), 2.88-2.91 (m, 2H), 3.85 (t, 2H, *J* = 6.6 Hz), 5.88 (bs, 1H), 6.97 (s, 1H), 7.01 (d, 1H, *J* = 8.0 Hz), 7.23 (d, 1H, *J* = 8.0 Hz); ¹³C NMR (CDCl₃): 14.0, 25.5, 26.6, 27.3, 28.4, 29.3, 31.8, 32.3 (2C), 37.6, 44.3, 48.1, 48.4, 48.8, 63.5, 125.4, 126.4, 129.6, 135.8, 136.5, 137.8, 178.2, ~219.0; LRMS for C₂₃H₃₀O₄ [M + H]⁺ 371.2 m/z.

benzyl 3-[3-(2-(benzyloxy)ethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]acetate (11)

To an ice-cooled solution of compound **2** (1.5 g, 3.86 mmol) in dry THF (20 mL) was added under argon LDA (1.8 M in THF, 2.8 mL, 5 mmol). The resulting deep yellow solution was stirring for 1 h then the ice-cooling bath was replaced by a dry ice-acetone bath. After 10 min, a solution of dry HMPA (0.77 mL, 5 mmol) and benzyl bromoacetate (0.79 mL, 5 mmol) in dry THF (5 mL) was added to the mixture. The cooling bath was then removed and the mixture stirred for 3 h before being quenched with a saturated aqueous solution of NH₄Cl. After EtOAc extractions, the combined organic phase was washed with water, brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude product

was purified by flash chromatography (EtOAc/hexanes 5:95) to afford 0.77 g of recovered ketone (30%) followed by 1.4 g (42%) of 16-alkylated product as a mixture of two 16 α /16 β diastereoisomer in proportions 90:10. Data only reported for major 16 α -diastereoisomer. ¹H NMR (CDCl₃): 0.97 (s, 3H), 1.43-2.43 (m, 13H), 2.85-2.90 (m, 4H), 2.93-3.03 (m, 1H), 3.71 (t, 2H, *J* = 7.3 Hz), 4.54 (s, 2H), 5.15 (s, 2H), 6.96 (s, 1H), 7.01 (d, 1H, *J* = 8.0 Hz), 7.20 (d, 1H, *J* = 8.0 Hz), 7.28-7.38 (m, 10H); ¹³C NMR (CDCl₃): 14.5, 25.5, 26.3, 27.7, 29.3, 31.5, 35.5, 35.7, 38.1, 40.9, 44.2, 47.7, 48.2, 66.5, 71.2, 72.9, 125.3, 126.3, 127.5, 127.6 (2C), 128.2 (2C), 128.3 (3C), 128.5 (2C), 129.5, 135.7, 136.3 (2C), 137.5, 138.4, 172.2, ~219.0. To an ice-cooled solution of above 16-alkylated steroid (769 mg, 1.4 mmol) in dry THF (20 mL) was slowly added under argon commercial LDA (1.8 M in THF, 0.6 mL, 14 mmol). After 1 h of stirring, the yellowish solution, which gradually turns brown, was cooled at -78°C. Dry MeOH (0.6 mL, 14 mmol) in THF (5 mL) was slowly added and the resulting mixture was stirred at -78°C for 1 h before to be quenched with a saturated aqueous solution of NH₄Cl. After EtOAc extractions, the combined organic phase was washed with water, brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography using EtOAc/hexanes (5:95) to afford (301 mg, 39%) of 16 β -alkylated compound **11** which contains 12% of 16 α -diastereoisomer.

¹H NMR (CDCl₃): 0.86 (s, 3H), 1.16-2.67 (m, 13H), 2.86-2.93 (m, 4H), 2.96 (dd, 1H, *J*₁ = 4.0 and *J*₂ = 16.0 Hz), 3.69 (t, 2H, *J* = 7.3 Hz), 4.54 (s, 2H), 5.16 (s, 2H), 6.97 (s, 1H), 7.03 (d, 1H, *J* = 8.0 Hz), 7.23 (d, 1H, *J* = 8.0 Hz), 7.25-7.38 (m, 10H); ¹³C NMR (CDCl₃): 14.2, 25.5, 26.6, 28.7, 29.2, 31.8, 35.7, 36.4, 37.6, 44.3, 45.6, 48.3, 49.1, 66.5, 71.2, 72.8, 125.2, 126.3, 127.5, 127.6 (2C), 128.2 (3C), 128.3 (2C), 128.5 (2C), 129.5, 135.7, 136.3 (2C), 137.5, 138.4, 172.0, ~219.0. LRMS for C₃₆H₄₀O₄ [M + H]⁺ 537.2 m/z.

3-[3-(2-hydroxyethyl)-17-oxoestra-1(10),2,4-trien-16 β -yl]acetic acid (**12**)

To a solution of compound **11** (301 mg, 0.56 mmol) in 1:1 EtOAc/MeOH mixture (10 mL) under an argon atmosphere was added palladium hydroxide on carbon (loading 20% weight, 30 mg). The flask was purged three times with hydrogen and stirred at room temperature for 1 h. The suspension was filtered over celite, washed with MeOH and evaporated under reduce pressure. The crude compound was purified by flash chromatography using MeOH/DCM (1:99 to 4:96) to give 108 mg (54%) of acid **12**.

¹H NMR (CDCl₃): 0.89 (s, 3H), 1.40-2.65 (m, 13H), 2.82 (t, 2H, *J* = 6.5 Hz), 2.88-2.99 (m, 3H), 3.86 (t, 2H, *J* = 6.5 Hz), 6.98 (s, 1H), 7.03 (d, 1H, *J* = 8.0 Hz), 7.24 (d, 1H, *J* = 8.0 Hz); ¹³C NMR (CDCl₃): 14.3, 25.6, 26.7, 28.8, 29.3, 31.9, 36.1, 37.6, 38.6, 44.4, 45.5, 48.5, 49.2, 63.7, 125.5, 126.5, 129.7, 135.9, 136.7, 137.8, 176.8, ~219.0; LRMS for C₂₂H₂₈O₄ [M + H]⁺ 357.2 m/z.

3-[3-(2-hydroxyethyl)-17-oxoestra-1(10),2,4-trien-16 β -yl]-N-(5-methyl-1,3-thiazol-2-yl)propanamide (**13**)

To a solution of compound **10** (255 mg, 0.7 mmol) in dry DMF (1 mL) was added HATU (364 mg, 0.96 mmol). After 10 min of stirring at room temperature, 5-methyl-1,3-thiazole (111 mg, 0.96 mmol) was added followed by DIPEA (366 μ L, 2.1 mmol). The yellow resulting mixture was stirred overnight at room temperature. After dilution with water and extraction with EtOAc, the organic phase was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography using acetone/hexanes (3:7) to give 195 mg (60%) of amide **13** as a foam.

¹H NMR (CDCl₃): 0.90 (s, 3H), 1.38-2.38 (m, 14H), 2.41 (d, 3H, *J* = 1.1 Hz), 2.71 (t, 2H, *J* = 7.1 Hz), 2.82 (t, 2H, *J* = 6.5 Hz), 2.87-2.92 (m, 2H), 3.86 (t, 2H, *J* = 6.5 Hz), 6.97 (s, 1H), 7.00 (d, 1H, *J* = 8.0 Hz), 7.12 (d, 1H, *J* = 1.2 Hz), 7.23 (d, 1H, *J* = 8.0 Hz); ¹³C NMR (MeOD): 11.3, 14.5, 26.9, 27.9, 28.8, 29.4, 30.4, 32.1, 34.8, 39.3, 39.8, 45.7, 49.7, 49.8, 50.1, 64.4, 126.2, 127.4, 128.6, 130.5, 135.4, 137.4 (2C), 138.8, 158.4, 172.8, ~219.0; LRMS for C₂₇H₃₅N₂O₃S [M + H]⁺ 467.2 m/z.

3-[3-(2-hydroxyethyl)-17-oxoestra-1(10),2,4-trien-16 β -yl]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (**14**)

Amide **14** was prepared as described for preparation of compound **13**. Thus, starting from acid **12** (61 mg, 0.17 mmol), we obtained 55 mg (71%) of amide **14** as a foam after chromatography using MeOH/DCM (0.5:99.5).

¹H NMR (CDCl₃) (57:43 mixture of 2 conformers): 0.62 and 0.90 (2s, 3H), 1.39-2.53 (m, 12H), 2.40 and 2.41 (2d, 3H, *J* = 1.0 Hz), 2.63-2.73 (m, 1H), 2.68-2.93 (m, 4H), 3.01-3.19 (m, 1H), 3.81-3.87 (m, 2H), 6.95 and 6.96 (2s, 1H), 6.98-7.03 (m, 1H), 7.09 and 7.14 (2d, 1H, *J* = 1.1 Hz), 7.22 (d, 1H, *J* = 8.0 Hz); ¹³C NMR (CDCl₃) (57:43 mixture of 2 conformers): 11.5, 11.6, 14.4, 14.6, 25.6, 26.4, 26.7, 27.4, 28.9, 29.3, 29.4, 31.9, 33.4, 33.6, 37.6, 38.2, 38.6, 38.7, 42.7, 43.5, 44.4, 45.9, 48.5, 49.2, 49.3, 50.7, 63.6, 125.5, 125.6, 126.3, 126.4, 127.5, 127.9, 129.6, 129.7, 133.6, 133.8, 135.8, 136.0, 136.6, 136.7, 137.8, 138.1, 156.1, 157.8, 169.2, 172.5, ~219.0; LRMS for C₂₆H₃₃N₂O₃S [M + H]⁺ 453.2 m/z.

3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16 β -yl]-N-(5-methyl-1,3-thiazol-2-yl)propanamide (**PBRM-III**)

PBRM-III was prepared as described for the preparation of compound **6**. Thus, bromination of amide **13** (195 mg, 0.42 mmol) gave 57 mg (26%) of **PBRM-III** as a solid after chromatography using acetone/DCM (5:95).

¹H NMR (Acetone-d₆): 0.91 (s, 3H), 1.39-2.45 (m, 14H), 2.38 (s, 3H), 2.69 (t, 2H, *J* = 7.2 Hz), 2.80-2.92 (m, 2H), 3.10 (t, 2H, *J* = 7.4 Hz), 3.66 (t, 2H, *J* = 7.4 Hz), 7.00 (s, 1H), 7.06 (d, 1H, *J* = 7.8 Hz), 7.07 (s, 1H), 7.26 (d, 1H, *J* = 7.8 Hz); ¹³C NMR (Acetone-d₆): 10.4, 13.5, 25.5, 26.6, 27.7, 28.2, 29.2, 32.0, 33.3, 33.7, 37.8, 38.7, 44.4, 48.1, 48.2, 48.7, 125.4, 126.0, 126.5, 129.2, 134.6, 136.3, 136.5, 138.3, 156.4, 170.2, ~219.0; LRMS for C₂₇H₃₄BrN₂O₂S [M + H]⁺ 529.1 and 531.5 m/z. HPLC purity = 94.8%.

3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (PBRM-IV)

PBRM-IV was prepared as described for preparation of compound 6. Thus, bromination of amide 14 (50 mg, 0.11 mmol) gave 25 mg (32%) of PBRM-IV as a white solid after chromatography using acetone/DCM (5:95).

¹H NMR (CDCl₃): 0.63 (s, 3H), 1.12-2.46 (m, 11H), 2.42 (s, 3H), 2.63-2.73 (m, 1H), 2.83-2.93 (m, 2H), 3.04-3.16 (m, 3H), 3.55 (t, 2H, *J* = 7.6 Hz), 5.93 (bs, 1H), 6.93 (s, 1H), 6.99 (d, 1H, *J* = 7.7 Hz), 7.09 (s, 1H), 7.23 (d, 1H, *J* = 7.9 Hz); ¹³C NMR (CDCl₃): 11.5, 14.6, 26.4, 27.4, 29.4, 33.0, 33.4, 33.6, 38.6, 38.7, 39.0, 42.7, 43.6, 49.3, 50.7, 125.6, 126.0, 128.0, 129.2, 133.8, 136.3, 136.8, 138.6, 156.1, 172.5, ~219.0; LRMS for C₂₆H₃₂BrN₂O₂S [M + H]⁺ 515.1 and 517.1 m/z. HPLC purity = 99.9%.

3-[3-(2-bromoethyl)-17β-hydroxyestra-1(10),2,4-trien-16β-yl]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (PBRM-V)

PBRM-V was prepared as described for preparation of PBRM-XII. Thus, reduction of PBRM-III (50 mg, 0.09 mmol) with NaBH₄ gave 10.3 mg (20%) of PBRM-V as a white solid after recrystallization (MeOH).

¹H NMR (CDCl₃): 0.80 (s, 3H), 1.02-2.38 (m, 12H), 2.41 (s, 3H), 2.53-2.63 (m, 2H), 2.83-2.87 (m, 2H), 3.10 (t, 2H, *J* = 7.8 Hz), 3.55 (t, 2H, *J* = 7.6 Hz), 3.81 (d, 1H, *J* = 9.2 Hz), 6.93 (s, 1H), 6.99 (d, 1H, *J* = 8.0 Hz), 7.08 (s, 1H), 7.25 (d, 1H, *J* = 8.0 Hz); ¹³C NMR (CDCl₃): 11.6, 12.4, 26.0, 27.4 (2C), 29.5, 32.6, 33.0, 35.6, 37.6, 38.0, 39.0, 39.5, 44.2, 44.3, 48.7, 82.0, 125.6, 125.9, 126.6, 129.2, 133.6, 136.2, 136.9, 138.9, 157.4, 170.9. LRMS for C₂₇H₃₆BrN₂O₂S [M + H]⁺ 531.1 and 533.1 m/z. HPLC purity = 96.3%.

3-[3-(2-hydroxyethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]propanamide (15)

30

To a solution of compound 10 (74 mg, 0.2 mmol) in dry DMF (0.2 mL) was added HATU (114 mg, 0.3 mmol). After 10 min of stirring at room temperature, 1-(pyridin-3-yl)methanamine (32 mg, 0.3 mmol) was added followed by DIPEA (103 μL, 0.4 mmol). The

yellow resulting mixture was stirred overnight at room temperature. After dilution with water and extraction with EtOAc, the organic phase was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was purified by chromatography using MeOH/DCM (4:96) to give 70 mg (70%) of amide **13** as a foam.

- 5 ¹H NMR (Acetone-d₆): 0.85 (s, 3H), 1.32-2.30 (m, 13H), 2.35-2.42 (m, 3H), 2.75 (t, 2H, *J* = 7.0 Hz), 2.83-2.86 (m, 2H), 3.74 (t, 2H, *J* = 7.0 Hz), 4.43 (d, 2H, *J* = 5.9 Hz), 6.95 (s, 1H), 6.99 (d, 1H, *J* = 8.0 Hz), 7.19 (d, 1H, *J* = 7.9 Hz), 7.29-7.33 (m, 1H), 7.71 (d, 1H, *J* = 7.6 Hz), 7.82 (bt, 1H), 8.46 (bs, 1H), 8.56 (bs, 1H). ¹³C NMR (Acetone-d₆): 13.6, 25.6, 26.6, 28.2, 28.3, 29.2, 32.0, 34.2, 37.8, 39.0, 40.3, 44.4, 48.1, 48.4, 48.8, 63.1, 123.4, 125.1, 126.3, 129.5, 135.1, 135.4, 136.1, 136.7, 137.4, 148.2, 149.2, 172.1, ~219.0; LRMS for C₂₉H₃₇N₂O₃ [M + H]⁺ 461.2 m/z.
- 10

3-[3-(2-hydroxyethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]acetamide (**16**)

- Amide **16** was prepared as described for preparation of compound **15**. Thus, starting from acid **12** (45 mg, 0.13 mmol), we obtained 35 mg (57%) of amide **15** as a foam after chromatography using MeOH/DCM (4:96).
- 15

- ¹H NMR (CDCl₃): 0.82 (s, 3H), 1.35-2.46 (m, 12H), 2.52-2.65 (m, 1H), 2.71 (dd, 1H, *J*₁ = 5.6 and *J*₂ = 14.6 Hz), 2.80 (t, 1H, *J* = 6.6 Hz), 2.84-2.92 (m, 2H), 3.83 (t, 2H, *J* = 6.6 Hz), 4.43 (t, 2H, *J* = 5.3 Hz), 6.95-7.02 (m, 3H), 7.20 (d, 1H, *J* = 8.0 Hz), 7.26 (d, 1H, *J* = 7.5 Hz), 7.64 (d, 1H, *J* = 7.6 Hz), 8.46-8.50 (m, 2H); ¹³C NMR (CDCl₃): 14.2, 25.5, 26.6, 29.0, 29.2, 31.8, 37.5, 38.3, 38.6, 41.0, 44.2, 46.1, 48.5, 48.9, 63.5, 123.5, 125.3, 126.3, 129.6, 134.0, 135.6, 136.0, 136.5, 137.6, 148.6, 149.0, 171.3, ~219.0; LRMS for C₂₈H₃₅N₂O₃ [M + H]⁺ 447.2 m/z.
- 20

3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]propanamide (**PBRM-VII**)

25

PBRM-VII was prepared as described for preparation of compound **6**. Thus, bromination of amide **15** (63 mg, 0.12 mmol) gave 24 mg (34%) of **PBRM-VII** as a yellow solid after chromatography using acetone/diethyl ether/DCM (4:20:80).

- ¹H NMR (Acetone-d₆): 0.88 (s, 3H), 1.30-2.35 (m, 12H), 2.39 (t, 2H, *J* = 7.1 Hz), 2.83-2.90 (m, 3H), 3.10 (t, 2H, *J* = 7.5 Hz), 3.66 (t, 2H, *J* = 7.5 Hz), 4.43 (d, 2H, *J* = 6.0 Hz), 7.01 (s, 1H), 7.05 (d, 1H, *J* = 8.2 Hz), 7.25 (d, 1H, *J* = 8.0 Hz), 7.31 (dd, 1H, *J* = 4.5 and 7.7 Hz), 7.71 (d, 1H, *J* = 7.8 Hz), 8.46 (d, 1H, *J* = 3.9 Hz), 8.55 (bs, 1H); ¹³C NMR (Acetone-d₆): 13.5, 25.5, 26.5, 28.2, 28.4, 29.1, 32.0, 33.3, 34.1, 37.7, 38.7, 40.2, 44.4, 48.1, 48.3, 48.8,
- 30

123.2, 125.4, 126.0, 129.2, 135.0, 135.3, 136.3, 136.5, 138.3, 148.3, 149.2, 171.9, ~219.0; LRMS for $C_{29}H_{36}BrN_2O_2$ $[M + H]^+$ 523.2 and 525.2 m/z. HPLC purity = 71.1%.

3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16 β -yl]-N-[(pyridin-3-yl)methyl]acetamide (PBRM-VIII)

- 5 **PBRM-VIII** was prepared as described for preparation of compound **6**. Thus, bromination of amide **16** (34 mg, 0.07 mmol) gave 19 mg (50%) of **PBRM-VIII** as a yellow solid after chromatography (acetone/ether/DCM (3:20:80)).

1H NMR ($CDCl_3$): 0.84 (s, 3H), 1.38-2.42 (m, 12H), 2.56-2.65 (m, 1H), 2.72 (dd, 1H, J = 5.8 and 14.6 Hz), 2.88-2.92 (m, 2H), 3.10 (t, 2H, J = 7.6 Hz), 3.55 (t, 2H, J = 7.6 Hz), 4.45 (d, 2H, J = 6.0 Hz), 6.73 (t, 1H, J = 5.5 Hz), 6.94 (s, 1H), 6.99 (d, 1H, J = 8.0 Hz), 7.23 (d, 2H, J = 8.1 Hz), 7.65 (d, 1H, J = 7.8 Hz), 8.53 (bs, 2H); ^{13}C NMR ($CDCl_3$): 14.3, 25.6, 26.7, 29.3, 31.9, 32.9 (2C), 37.6, 38.4, 39.0, 41.1, 44.4, 46.2, 48.6, 49.1, 123.6, 125.5, 126.0, 129.3, 135.0, 135.6, 136.4, 136.7, 138.3, 148.8, 149.2, 171.3, ~219.0; LRMS for $C_{28}H_{34}BrN_2O_2$ $[M + H]^+$ 509.2 and 511.2 m/z. HPLC purity = 92.2%.

- 15 3-[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-16 β -yl]-N-[(pyridin-3-yl)methyl]propanamide (PBRM-IX)

PBRM-IX was prepared as described for preparation of **PBRM-XII**. Thus, reduction of **PBRM-VII** (17 mg, 0.03 mmol) with $NaBH_4$ gave 13 mg (76%) of **PBRM-IX** as a white foam after chromatography using MeOH/DCM (8:92).

- 20 1H NMR (Acetone- d_6): 0.81 (s, 3H), 1.01-2.41 (m, 14H), 2.80-2.90 (4H masked), 3.10 (t, 2H, J = 7.4 Hz), 3.65 (t, 2H, J = 7.5 Hz), 3.74 (d, 1H, J = 9.5 Hz), 4.43 (d, 2H, J = 6.0 Hz), 6.98 (s, 1H), 7.04 (d, 1H, J = 8.3 Hz), 7.22-7.33 (m, 2H), 7.60 (bs, 1H), 7.71 (d, 1H, J = 7.8 Hz), 8.47 (s, 1H), 8.55 (s, 1H); ^{13}C NMR ($DMSO-d_6$): 12.6, 25.7, 27.0, 28.1, 29.0, 31.8, 34.5, 34.9, 37.4, 37.8 (2C), 38.0, 43.6 (2C), 43.8, 48.2, 80.3, 123.4, 125.2, 125.9, 128.9, 135.0, 25 ^{13}C NMR ($DMSO-d_6$): 12.6, 25.7, 27.0, 28.1, 29.0, 31.8, 34.5, 34.9, 37.4, 37.8 (2C), 38.0, 43.6 (2C), 43.8, 48.2, 80.3, 123.4, 125.2, 125.9, 128.9, 135.0, 135.3, 135.8, 136.1, 138.5, 147.9, 148.7, 172.7; LRMS for $C_{29}H_{38}BrN_2O_2$ $[M + H]^+$ 525.1 and 527.1 m/z. HPLC purity = 91.4%.

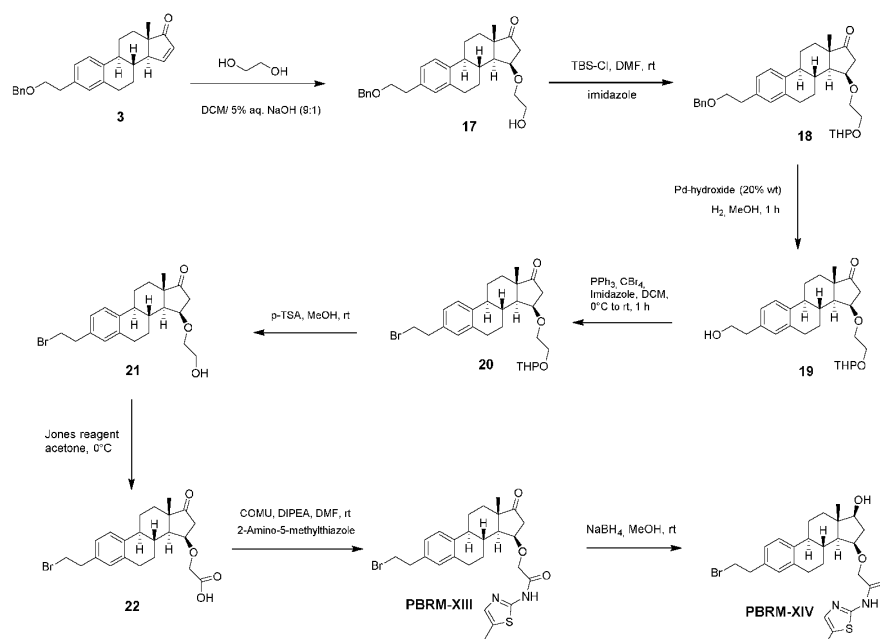
3-[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-16 β -yl]-N-[(pyridin-3-yl)methyl]acetamide (PBRM-X)

- PBRM-X** was prepared as described for preparation of **PBRM-XII**. Thus, reduction of **PBRM-VIII** (10 mg, 0.07 mmol) with $NaBH_4$ gave 3.3 mg (33%) of **PBRM-X** as a white foam after chromatography using MeOH/DCM (8:92).

1H NMR ($CDCl_3$): 0.79 (s, 3H), 1.08-2.38 (m, 12H), 2.56-2.66 (m, 1H), 2.77 (dd, 1H, J_1 = 10.6 and J_2 = 14.7 Hz), 2.83-2.88 (m, 2H), 3.10 (t, 2H, J = 7.7 Hz), 3.55 (t, 2H, J = 7.6 Hz),

- 3.88 (d, 1H, $J = 9.6$ Hz), 4.46 (d, 2H, $J = 6.0$ Hz), 6.22 (t, 1H, $J = 5.4$ Hz), 6.92 (s, 1H), 7.01 (d, 1H, $J = 8.0$ Hz), 7.23-7.32 (m, 2H), 7.64 (d, 1H, $J = 7.9$ Hz), 8.54 (bs, 2H); ^{13}C NMR (CDCl_3): 12.7, 26.0, 27.5, 29.5, 29.7, 32.8, 33.0, 37.8, 37.9, 38.6, 39.0, 41.2, 44.2, 44.3, 49.2, 80.8, 123.7, 125.6, 125.9, 129.2, 135.6 (2C), 136.1, 136.9, 139.0, 148.9, 149.1, 174.5;
- 5 LRMS for $\text{C}_{28}\text{H}_{36}\text{BrN}_2\text{O}_2$ $[\text{M} + \text{H}]^+$ 511.1 and 513.1 m/z . HPLC purity = 94.8%.

Chemical synthesis of PBRM-XIII and PBRM-XIV



Scheme 6. Synthesis of PBRM-XIII and PBRM-XIV from intermediate compound 3. Reagents and conditions.

3-[2-(benzyloxy)ethyl]-15 β -(2-hydroxyethoxy)estra-1(10),2,4-trien-17-one (17)

To a solution of compound 3 (700 mg, 1.81 mmol) in DCM (15 mL) was added ethylene glycol (15 mL) and 1 mL of an NaOH (5%) aqueous solution. The solution was vigorously stirred at room temperature for 72 h. The resulting solution was diluted with DCM and was washed with water. The organic layer was dried over a cotton plug and evaporated under reduced pressure. The crude compound was purified by flash chromatography using EtOAc/hexanes (2:8 to 10:0) to give 580 mg (72%) of compound 17.

^1H NMR (CDCl_3): 1.19 (s, 3H), 1.40-2.47 (m, 12H), 2.91 (m, 4H), 3.45 (m, 1H), 3.70 (m, 5H), 4.26 (t, 1H, $J = 5.4$ Hz), 4.56 (s, 2H), 7.00 (s, 1H), 7.04 (d, 1H, $J = 8.0$ Hz), 7.23 (d, 1H, $J = 8.0$ Hz), 7.29-7.39 (m, 5H); ^{13}C NMR (CDCl_3): 17.6, 25.6, 26.3, 29.2, 32.7, 34.8,

35.8, 43.2, 44.5, 47.3, 54.6, 62.0, 70.8, 71.3, 73.0, 75.3, 125.2, 126.4, 127.6, 127.7 (2C), 128.4 (2C), 129.6, 136.4 (2C), 137.7, 138.5, 219.3; LRMS for C₂₉H₃₇O₄ [M + H]⁺ 449.2 m/z.

3-[2-(benzyloxy)ethyl]-15β-[2-[(oxan-2-yl)oxy]ethoxy]estra-1(10),2,4-trien-17-one (18)

To a solution of compound **17** (580 mg, 1.29 mmol) at 0°C in DCM (15 mL) was added 3,4-dihydro-2H-pyran (544 mg, 6.46 mmol) and *p*-toluenesulfonic acid monohydrate (*p*-TSA) (3 mg, 0.015 mmol). The solution was stirred at 0°C for 10 min and then allowed to return at room temperature and stirred for 1 h. The solution was then poured into a saturated sodium bicarbonate solution and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude compound was purified by flash chromatography using EtOAc/hexanes (3:7 with 1% TEA) to give 580 mg (84%) of compound **18**.

¹H NMR (Acetone-d₆): 1.17 and 1.18 (2s, 3H), 1.38-2.50 (m, 16H), 2.70-2.98 (m, 5H), 3.44 (m, 1H), 3.55 (m, 2H), 3.68 (t, 2H, *J* = 7.0 Hz), 3.82 (m, 3H), 4.32 (t, 1H, *J* = 5.4 Hz), 4.53 (s, 2H), 4.67 (m, 1H), 6.99 (s, 1H), 7.03 (d, 1H, *J* = 8.0 Hz), 7.22 (d, 1H, *J* = 8.0 Hz), 7.33 (m, 5H); ¹³C NMR (Acetone-d₆): 17.0, 19.0, 25.4, 25.6, 26.0, 29.1, 30.5, 32.8, 35.0, 35.6, 42.7, 44.5, 46.9, 54.2, 61.0, 66.3, 68.9, 71.2, 72.3, 75.0, 98.1, 125.0, 126.2, 127.2, 127.4 (2C), 128.1 (2C), 129.4, 136.2, 136.5, 137.7, 139.1, 217.9.

3-(2-hydroxyethyl)-15β-[2-[(oxan-2-yl)oxy]ethoxy]estra-1(10),2,4-trien-17-one (19)

To a solution of compound **18** (500 mg, 0.94 mmol) in MeOH (30 mL) under an argon atmosphere was added palladium hydroxide on carbon (loading 20% weight) (50 mg). The flask was purged three times with hydrogen and stirred at room temperature for 1 h. The suspension was then filtered over celite, washed with MeOH and evaporated under reduced pressure. The crude compound was purified by flash chromatography using EtOAc/hexanes (1:1 to 7:3; with 1% TEA) to give 180 mg (45%) of compound **19**.

¹H NMR (Acetone-d₆): 1.17 and 1.18 (2s, 3H), 1.40-2.46 (m, 18H), 2.68-2.90 (m, 5H), 3.46 (m, 1H), 3.58 (m, 2H), 3.73 (m, 3H), 3.82 (m, 2H), 4.33 (t, 1H, *J* = 5.4 Hz), 4.67 (m, 1H), 6.97 (s, 1H), 7.00 (d, 1H, *J* = 7.9 Hz), 7.21 (d, 1H, *J* = 7.9 Hz); ¹³C NMR (DMSO-d₆): 17.2, 18.8, 25.0, 25.3, 28.8, 30.2, 32.3, 34.6, 42.1, 44.0, 46.5, 53.3, 56.5, 60.7, 62.2, 65.8, 68.0, 74.5, 76.0, 97.6, 124.9, 126.2, 129.2, 135.7, 136.5, 137.0, 219.0; LRMS for C₂₇H₃₉O₅ [M + H + H₂O]⁺ 461.2 m/z.

3-(2-bromoethyl)-15β-[2-[(oxan-2-yl)oxy]ethoxy]estra-1(10),2,4-trien-17-one (20)

To a solution of compound **19** (170 mg, 0.41 mmol) in DCM (20 mL) at 0°C was added triphenylphosphine (202 mg, 0.77 mmol) and imidazole (212 mg, 3.11 mmol) followed by

the dropwise addition of carbon tetrabromide (256 mg, 0.77 mmol) in DCM (5 mL) over 2 min. The solution was then stirred for 1 h. The resulting solution was diluted with DCM (25 mL) and washed with a saturated solution of sodium bicarbonate. The organic layer was filtered over a cotton plug and evaporated under reduce pressure. The crude compound
5 was purified by flash chromatography using EtOAc/hexanes (1:9; with 1% TEA) to give 69 mg (36%) of compound **20**.

¹H NMR (Acetone-d₆): 1.17 and 1.18 (2s, 3H), 1.40-2.45 (m, 16H), 2.68-2.92 (m, 3H), 3.11 (t, 2H, *J* = 7.5 Hz), 3.46 (m, 1H), 3.56 (m, 2H), 3.66 (t, 2H, *J* = 7.5 Hz), 3.82 (m, 3H), 4.32 (t, 1H, *J* = 5.4 Hz), 4.66 (m, 1H), 7.02 (s, 1H), 7.06 (d, 1H, *J* = 7.9 Hz), 7.27 (d, 1H, *J* = 8.1 Hz); ¹³C NMR (Acetone-d₆): 17.0, 19.0, 25.4, 25.5, 25.9, 30.5, 32.8, 33.3, 34.9, 38.7, 42.7,
10 44.5, 46.9, 54.2, 60.9, 66.3, 68.7, 68.9, 75.0, 98.1, 125.2, 126.0, 129.2, 136.4, 136.5, 138.5, 217.9; LRMS for C₂₇H₃₈BrO₄ [M + H + 2 H₂O]⁺ 536.1 and 538.1 m/z.

3-(2-bromoethyl)-15β-(2-hydroxyethoxy)estra-1(10),2,4-trien-17-one (21)

To a solution of compound **20** (65 mg, 0.13 mmol) in 3 mL of a mixture of DCM/MeOH (9:1)
15 at 0°C was added *p*-TSA (2 mg, 0.01 mmol). The solution was stirred at this temperature for 30 min. The resulting solution was diluted with DCM and then washed with a saturated solution of sodium bicarbonate. The organic phase was filtered over a cotton plug and evaporated under reduce pressure. The crude compound was purified by flash chromatography using EtOAc/hexanes (1:1 to 1:0) to give 35 mg (65%) of compound **21**.

20 ¹H NMR (Acetone-d₆): 1.16 (s, 3H), 1.40-2.46 (m, 10H), 2.67-2.95 (m, 3H), 3.11 (t, 2H, *J* = 7.5 Hz), 3.47 (m, 1H), 3.51-3.70 (m, 6H), 4.31 (t, 1H, *J* = 5.4 Hz), 7.02 (s, 1H), 7.06 (d, 1H, *J* = 8.0 Hz), 7.26 (d, 1H, *J* = 7.9 Hz); ¹³C NMR (Acetone-d₆): 17.1, 25.5, 26.0, 29.2, 32.8, 33.4, 34.7, 38.7, 42.9, 44.4, 46.8, 54.3, 61.3, 71.3, 75.0, 125.2, 126.0, 129.2, 136.3, 136.6, 138.6, 217.9; LRMS for C₂₂H₃₀BrO₃ [M + H]⁺ 421.1 and 423.1 m/z.

25 {[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15β-yl]oxy}acetic acid (22)

To a solution of compound **21** (33 mg, 0.08 mmol) in acetone (3 mL) at 0°C was dropwise added Jones reagent (100 μL) and the solution was stirred for 2 h at this temperature. 2-propanol was then added and stirred for an additional 10 min at 0°C. The resulting solution was poured into water, extracted with EtOAc, washed with brine, dried with sodium sulfate,
30 filtered and evaporated under reduce pressure to give 35 mg of carboxylic acid **22** as single product The compound was used for the next step without further purification.

¹H NMR (CDCl₃): 1.20 (s, 3H), 1.36-2.46 (m, 11H), 2.70-2.99 (m, 3H), 3.11 (t, 2H, *J* = 7.6 Hz), 3.55 (t, 2H, *J* = 7.7 Hz), 4.15 (q of AB system, 2H, *J*_A = 7.0 Hz, *J*_B = 16.7 Hz), 4.37 (t, 1H, *J* = 5.1 Hz), 6.96 (s, 1H), 7.00 (d, 1H, *J* = 8.0 Hz), 7.25 (d, 1H, *J* = 8.8 Hz); ¹³C NMR

(CDCl₃): 17.4, 25.4, 26.1, 29.1, 32.7, 33.0, 34.6, 39.0, 42.9, 44.6, 47.3, 54.6, 66.4, 76.2, 125.4, 126.0, 129.2, 136.4, 136.8, 138.4, 174.4, ~218.0; LRMS for C₂₂H₂₈BrO₄ [M + H]⁺ 435.1 and 437.1 m/z.

2-[[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15 β -yl]oxy]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (PBRM-XIII)

To a solution of compound **21** (32 mg, 0.073 mmol) in anhydrous DMF (2 mL) was added (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (35 mg, 0.08 mmol) and the mixture was stirred 5 min before the addition of 2-amino-5-methylthiazole (17 mg, 0.15 mmol) and diisopropylethylamine (DIPEA) (26 μ L, 0.15 mmol). The resulting solution was stirred at room temperature for 2 h. The solution was then poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude compound was purified by flash chromatography using EtOAc/hexanes (1:1) to give 25 mg (65%) of **PBRM-XIII**.

¹H NMR (Acetone-d₆): 1.25 (s, 3H), 1.30-2.53 (m, 13H), 2.39 (d, 3H, *J* = 1.2 Hz), 2.81-3.06 (m, 3H), 3.11 (t, 2H, *J* = 7.5 Hz), 3.66 (t, 2H, *J* = 7.5 Hz), 4.34 (q of AB system, 2H, *J*_A = 22.5 Hz, *J*_B = 7.3 Hz), 4.56 (t, 1H, *J* = 5.3 Hz), 7.02 (s, 1H), 7.07 (d, 1H, *J* = 8.0 Hz), 7.09 (d, 1H, *J* = 1.2 Hz), 7.28 (d, 1H, *J* = 7.9 Hz), 10.0 (s, 1H); ¹³C NMR (Acetone-d₆): 10.5, 17.1, 25.4, 26.1, 29.0, 32.9, 33.4, 34.8, 38.7, 42.4, 44.6, 46.8, 54.0, 68.2, 76.4, 125.3, 126.0, 127.3, 129.2, 134.9, 136.4, 136.6, 138.4, 155.2, 167.2, 217.0; LRMS for C₂₆H₃₂BrN₂O₃S [M + H]⁺ 531.1 and 533.1 and m/z. HPLC purity = 99.0%.

2-[[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-15 β -yl]oxy]-N-(5-methyl-1,3-thiazol-2-yl)acetamide (PBRM-XIV)

To a solution of **PBRM-XIII** (15 mg, 0.028 mmol) in MeOH/DCM (9:1) (2 mL) was added sodium borohydride (3.2 mg, 0.08 mmol). The resulting solution was stirred at 0°C for 1 h before to be poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure to give 13 mg (87%) of **PBRM-XIV**.

¹H NMR (Acetone-d₆): 0.90 (m, 1H), 1.10 (s, 3H), 1.20-2.543 (m, 11H), 2.39 (d, 3H, *J* = 1.2 Hz), 2.80-3.04 (m, 3H), 3.10 (t, 2H, *J* = 7.5 Hz), 3.66 (t, 2H, *J* = 7.5 Hz), 3.72 (t, 1H, *J* = 8.5 Hz), 4.15 (m, 1H), 4.18 (q of AB system, 2H, *J*_A = 57.0 Hz, *J*_B = 15.4 Hz), 7.00 (s, 1H), 7.05 (d, 1H, *J* = 8.0 Hz), 7.08 (d, 1H, *J* = 1.2 Hz), 7.28 (d, 1H, *J* = 8.0 Hz), 9.72 (s, 1H); ¹³C NMR (Acetone-d₆): 10.5, 13.4, 25.9, 26.7, 29.5, 33.3, 35.0, 38.0, 38.7, 39.0, 42.7, 44.6, 54.4,

67.4, 78.2, 80.4, 125.3, 125.9, 127.3, 129.2, 134.9, 136.3, 136.7, 138.9, 155.0, 167.3; LRMS for $C_{26}H_{34}BrN_2O_3S$ $[M + H]^+$ 533.1 and 535.1 m/z. HPLC purity = 99.8%.

Biological tests

I. Inhibition potency

5 *17 β -HSD1 inhibition assay (Method A):*

T-47D breast cancer cells (purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA)) were grown in RPMI medium supplemented with 5% (v/v) fetal bovine serum (FBS) treated with dextran-coated charcoal, L-glutamine (2 nM), penicillin (100 IU/mL), streptomycin (100 μ g/mL) and insulin (50 ng/mL).

- 10 The cells were seeded in a 24-well plate (8 000-25 000 cells/well). Stock solution of each compound to be tested was previously prepared in dimethylsulfoxide (DMSO) and diluted with culture medium to achieve the appropriate concentrations prior to use. After 24 h of incubation, a diluted solution of each compound was added to the cells to obtain the appropriate final concentration from 0.5 nM to 1 μ M for IC₅₀ value determination. The final
15 concentration of DMSO in the well was adjusted to 0.1%.

These treatments were then preincubated for 1 h at 37°C. Additionally, a solution containing [³H]-E1 (7 nM) and cold E1 (53 nM) was added to obtain a total concentration in E1 of 60 nM.

- Cells were then incubated for 24 h, and each inhibitor was assessed in triplicate. After
20 incubation, the culture medium was removed and steroids were extracted with diethyl ether. The organic phase was evaporated to dryness with nitrogen. Residues were dissolved in DCM, dropped on silica gel thin layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA), and eluted with toluene/acetone (4:1) as solvent system. Substrate [³H]-E1/E1 and metabolite [³H]-E2/E2 were identified by comparison with reference
25 steroids (E1 and E2) and isolated. Labeled steroids were quantified with scintillation counter. The percentage of transformation and the percentage of inhibition were calculated as follow: % transformation = $100[3H]\text{-}E2 / ([3H]\text{-}E1 + [3H]\text{-}E2)$ and % of inhibition = $100(\%$ transformation without inhibitor – % transformation with inhibitor)/% transformation without inhibitor. The concentration inhibiting 50% of E1 to E2 transformation (IC₅₀) was
30 determined using GraphPad Prism 6 software.

Table 1. Concentration inhibiting 50% of the transformation of E1 to E2 by 17β-HSD1 in T-47D cells

Compound	IC ₅₀ (nM) (mean)
PBRM	138
PBRM-II	5.25

Conclusion: the compound of the invention, PBRM-II, incorporating the particular amide moiety at C15, is 26-fold more active than the compound of the prior art PBRM (2 experiments).

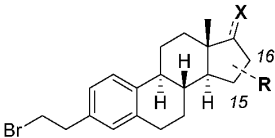
17β-HSD1 inhibition assay (Method B):

T-47D cells were grown in a medium supplemented with insulin (50 ng/mL) and 5% dextran-coated charcoal-treated FBS, which was used rather than untreated 10% FBS, to remove the remaining steroid hormones. Stock solution of each compound to be tested was previously prepared in DMSO and diluted with a culture medium to achieve the appropriate concentrations, prior to use. For the assay, the cells (3000 cells/well) were seeded and after 24 h of incubation, a diluted solution of each inhibitor was added to the cells to obtain appropriate final concentration (0.01, 0.1, 1, 5, 10, 50, 100, 500 and 1000 nM). The final concentration of DMSO in the well was adjusted to 0.1%. The inhibitor and cells were preincubated for 2 h at 37°C and a solution containing [14C]-E1 (10 nM) and cold E1 (50 nM) was added to obtain a final concentration of 60 nM. Cells were incubated for 24 h and each inhibitor was assessed in triplicate. After incubation, the culture medium was removed, and the steroids (labelled and unlabelled E1 and E2) were extracted with diethyl ether. The organic phase was evaporated to dryness with nitrogen. Residues were dissolved in DCM, spotted on silica gel TLC plates (EMD Chemicals Inc., Gibbstown, NJ, USA) and eluted with toluene/acetone (4:1) as the solvent system. Substrate [14C]-E1/E1 and metabolite [14C]-E2/E2 were identified by comparison with reference steroids (E1 and E2) and isolated. Radioactivity associated with each steroids was quantified using a Storm 860 Molecular Imager system (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation and of inhibition were calculated as follows: % transformation = 100 [14C]-E2 / ([14C]-E1 + [14C]-E2) and % inhibition = 100 (% transformation without inhibitor – % transformation with inhibitor)/% transformation without inhibitor. The concentration inhibiting 50% of E1 to E2 transformation (IC50) was determined using GraphPad Prism 6 software.

Table 2. Concentration inhibiting 50% of the transformation of E1 to E2 by 17β-HSD1 in T-47D cells

Compound	IC ₅₀ (nM) (mean)
PBRM	47
PBRM-II	2.05

5



#	R (Position)	R (Side Chain)	X	IC ₅₀ (nM) ¹	Efficacy vs PBRM ²
PBRM	16β	-CH ₂ -Phenyl- <i>m</i> -CONH ₂	β-OH	47.2 ± 2.7	--
PBRM-II	15β	-CH ₂ CH ₂ CONH-	O	3.8 ± 0.8	13.3
PBRM-II-OH	15β	-CH ₂ CH ₂ CONH-	β-OH	7.4 ± 0.2	6.8
PBRM-VIII	16β	-CH ₂ CONHCH ₂ -	O	39 ± 24	1.3
PBRM-X	16β	-CH ₂ CONHCH ₂ -	β-OH	11.4 ± 0.3	4.4
PBRM-XI	15β	-CH ₂ CH ₂ CONHCH ₂ -	O	16.9 ± 1.4	3.0
PBRM-XII	15β	-CH ₂ CH ₂ CONHCH ₂ -	β-OH	9.6 ± 0.5	5.3
CC-156	16β	----	β-OH	48.7 ± 16.7	1.0

¹ For the transformation of estrone (60 nM) to estradiol by 17β-HSD1 in T47D cells (n = 2); ² IC₅₀ (PBRM) / IC₅₀ (compound).

Conclusion: the compound of the invention, PBRM-II, incorporating the particular amide moiety at C15, is 23-fold more active than the compound of the prior art PBRM (2 experiments). Furthermore, PBRM-II (IC_{50} = 3.8 nM) is the most active 17β -HSD1 inhibitor from the series of PBRM analogs tested, followed by PBRM-II-OH, PBRM-XII, PBRM-X and
5 PBRM-XI (IC_{50} = 7.4, 9.6, 11.4 and 16.9 nM, respectively). All PBRM analogs tested were found to be more active than PBRM. Compound CC-156 was found to have a similar activity to PBRM.

Irreversibility of Inhibitors

T47-D cells were cultivated as mentioned above. Cells were pre-incubated with the
10 inhibitors for 2 h at 37°C, the medium was slowly removed and the cells were carefully washed 3 times with PBS (phosphate buffer saline). The remaining 17β -HSD1 activity was assayed as described above by incubating the cells with [^{14}C]-E1 (10 nM) and cold E1 (50 nM) for 24 h at 37°C. Then, subsequent quantification of the steroids in the supernatant using the procedure described above. The percentage of transformation and then the
15 percentage of inhibition were calculated as reported above.

Conclusion: Irreversibility of PBRM-2 has been confirmed since the inhibition activity was maintained after three washing steps (Fig. 3).

Additionally, the IC_{50} values without washing and with washing were determined. The concentration inhibiting 50% of E1 to E2 transformation (IC_{50}) was determined using
20 GraphPad Prism 6 software. The results are shown in Table 3 below.

Table 3. Irreversibility of 17β -HSD1 inhibitors PBRM and PBRM-II (E1 to E2 transformation)

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45				
17β-HSD1 Inhibitor	Inhibition Type	IC ₅₀ (nM) without washing	IC ₅₀ (nM) with washing	Efficacy lost after washing ¹
PBRM	Irreversible ²	47.2 ± 6.1	353 ± 117 ³	7.5
PBRM-II	Irreversible	3.2 ± 1.7	13.2 ± 10.7 ³	4.1
CC-156	Reversible ⁴	48.7 ± 16.7	8834	181

¹ IC₅₀ (with washing) / IC₅₀ (without washing); ² The irreversibility of PBRM was confirmed by X-ray analysis of a 17β-HSD1/PBRM complex showing a strong covalent bond between the enzyme and the inhibitor (Li et al. J. Phys. Chem. Lett. 2018, 9, 5275); ³ Data not significantly different from without washing. ⁴ The reversibility of CC-156 was previously confirmed by X-ray analysis of a 17β-HSD1/CC-156 complex showing no strong covalent bond between the enzyme and the inhibitor (Mazumdar et al. Biochem. J. 2009, 424, 357).

As seen from Table 3 above, the inhibition activity of PBRM-II was maintained after three washing steps, thus confirming that it acts as an irreversible inhibitor.

II. Antiproliferative activity of PBRM-II in T-47D cells

The ER⁺ breast cancer cell line T-47D was chosen because they express the ER, the predominantly ERα, and they proliferate in the presence of estrogenic compounds

The cell line was maintained in culture flasks (175 cm² growth area, BD Falcon) at 37°C in a 5% CO₂ humidified atmosphere. The T-47D cells were grown in phenol red free RPMI 1640 medium supplemented with 10% fetal bovine serum (FB, penicillin (100 IU/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and 17β-estradiol (1 nM).

For the protocol, T-47D cells were suspended in RPMI supplemented with insulin (50 ng/mL), instead of 17β-estradiol, and 5% charcoal-stripped FBS to deprive the media of estrogens. The cells were plated in 96-well plates at a density of 3 000 cells/well and allowed to attach for 48 h.

After this pre-incubation, the inhibitors and the reference compounds diluted in fresh culture media were added to the wells and replaced every 2 days for 7 days of treatment.

CellTiter 96® Aqueous One Solution Cell Proliferation Assay was used as an indirect colorimetric measurement of cell proliferation according to the manufacturer's instructions.

Briefly, after the treatments, 20 μ L of MTS solution was added to each well (100 μ L) of the plates and incubated at 37°C for 4 h.

The absorbance at 490 nm was then measured with a Thermo max microplate reader (Molecular Devices, Sunnyvale, CA). The control (culture media + DMSO) was set to 100% of cell proliferation. Results are provided in Fig. 1.

Conclusion: PBRM-II is not estrogenic and more efficient than PBRM to reduce the cell proliferation of estrogen-dependent T-47D cells.

III. Stability of PBRM-II in human liver microsomes

The study of the compounds in human liver microsomes is a useful predictive tool to evaluate its stability, as well as the potential metabolites.

Stability assays were performed for 1 h at 37°C, with or without 10 mM NADPH in the presence of 40 μ g of human liver S9 fraction from Corning (Melrose, MA, USA) and 10 μ M of substrate in a final 100 μ L volume of 50 mM Tris buffer supplemented with 10 mM $MgCl_2$.

Assays were ended by adding 100 μ L of methanol (MeOH), centrifuged at 13,000 g for 10 min to obtain a pellet of proteins.

The supernatant of two independent experiments were pooled and submitted to HPLC-MS analysis (Shimadzu LCMS-2020 APCI, Altima HP C18 (250 mm x 4.6 mm, 5 μ m) column).

The HPLC-MS run of 40 min was performed at flow rate of 1 mL/min with an elution gradient starting from 70:30 (MeOH/ H_2O) to 100% MeOH during first 15 min, followed by 10 min with MeOH 100% and a re-equilibration phase of 15 min with 70:30 (MeOH/ H_2O). Compounds were detected using APCI⁺ mode of ionization using full scan ionization between 100 and 1000 m/z. Remaining compound (expressed in %) was calculated by dividing the area under the curve of the substrate for the assays with NADPH by the one without NADPH and multiplied by 100. Values represent the average of two independent experiments (Fig. 2).

Conclusion: PBRM-II is remarkable more stable than PBRM and CC-156.

IV. Biodisponibility of PBRM-II compared to PBRM

Animals:

Six to seven week-old female Balb/c mice weighing approximately 19 g were obtained from Charles-River, Inc (St-Constant, Qc., Canada). The animals were acclimatized to

environmental conditions (temperature: $22 \pm 3^\circ\text{C}$; humidity: $50 \pm 20\%$; 12-h light/12-h dark cycles, lights on at 07:15 h) for at least 5 days before starting the experiment. The animals were housed three per cage and were allowed free access to water and a certified commercial rodent food (Rodent diet #T.2018.15, Harlan Teklad, Madison, Wisconsin, U.S.A.) and water were provided ad libitum. The experiments with animals were conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care. The study was performed in accordance with the CCAC Guide for Care and Use of Experimental Animals. Institutional approval was obtained.

10

Assay:

Study was carried out following oral administration of PBRM and PBRM-II at the following concentrations: 5, 15, 30 and 60 mg/kg of body weight in 0.1 mL of dimethylsulfoxide and sunflower oil (8:92). The compound was firstly dissolved in DMSO and thereafter we added sunflower oil to obtain a final concentration of DMSO of 8%. During this experiment, the mice were fasted from 8 h before administration of compounds. Blood samples for determination of plasma compound concentration were collected by cardiac puncture at 2 h post-dose from 3 mice per dose. Blood samples were collected into Microvette potassium-EDTA (ethylenediamine tetra-acetic acid)-coated tube (Sarstedt, Aktiegesellschaft & Co, Germany) and centrifuged at 3200 rpm for 10 minutes at 4°C . The plasma was collected and stored at -80°C until analyzed by liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) analysis.

Measurement of PBRM and PBRM-II in plasma:

The concentration of inhibitors was determined by LC/MS/MS analysis using a procedure developed at CHUQ (CHUL) - Research Center (Bioanalytical Service). For extraction from plasma, 100 μL of plasma sample is transferred to individual tubes and 600 μL of ammonium acetate (1 mM) is added. A methanolic solution (50 μL) containing the deuterated steroid internal standard is then added to each tube. Samples are transferred on Strata-X SPE columns (Phenomenex, Torrance, CA, USA) and each column is washed with water and methanol:water (10:90, v/v). The inhibitor is then eluted with 5 mL of methanol containing 1 mM ammonium acetate. Methanol is evaporated at 45°C under inert atmosphere and the dried residue reconstituted in 100 μL of methanol:water (85:15, v/v). For the inhibitor analysis, the HPLC system uses a 75 x 4.6-mm, reversed-phase phenyl-hexyl column (phenomenex, Torrance, USA) at a flow rate of 0.8 mL/min. The inhibitor is

detected using an API 4000 mass spectrometer, equipped with TurbolonSpray (Applied Biosystems, Canada). ESI in positive ion mode was used.

5 **Table 4.** 17β-HSD1 inhibition (IC₅₀) and inhibitor plasmatic concentration (IPC) obtained 2 h after a single oral (gavage) administration in mice at four doses

17β-HSD1 Inhibitor	IC ₅₀ ¹ (nM)	IPC-5 mg/kg ² (ng/mL)	IPC-15 mg/kg (ng/mL)	IPC-30 mg/kg (ng/mL)	IPC-60 mg/kg (ng/mL)
PBRM	47.2 ± 2.7	--	898 ± 89	1814 ± 130	3897 ± 468
PBRM-II	3.8 ± 0.8	231 ± 30	897 ± 55	2178 ± 125	3853 ± 205

¹ For the transformation of estrone (60 nM) to estradiol by 17β-HSD1 in T47D cells.

10 As can be seen from the data in Table 4 above, PBRM-II has a very similar oral biodisponibility (plasmatic concentration) than PBRM.

V. Biodistribution of PBRM-II in six tissues when injected orally (gavage) in mice

15 For this experiment, the mice were fasted from 10 h before inhibitors administration. A single dose of PBRM-II (30 mg/kg) was administered to female Balb/c mice (3 animals/time-point per dose) by gavage (PO) using Sunflower oil:DMSO (dimethylsulfoxide) (92:8) as vehicle. PBRM-II was first dissolved in DMSO and, thereafter, we added the appropriate co-solvent (SO) to obtain a final 8% concentration of DMSO when injecting 100 μL. Animals
20 were sacrificed by exsanguination under isoflurane 2, 6, 12 and 24 after administration of PBRM-II. Tissues (liver, kidney, uterus, ovary and brain) and the colon content were then collected and stored at -80 °C until the concentration of PBRM-II was determined by LC-MS/MS analysis using a procedure developed at CHU de Québec Research Center for steroid derivatives. The results are shown in Table 5 below and in Figure 4.

Table 5. Concentration of PBRM-II (ng) per g of six tissues

Time	Liver	±	Kidney	±	Uterus	±
2h	19776,1	9938,7	17714,7	9441,7	11860,1	15755,8
6h	16438,0	8662,9	10216,1	4206,0	26604,7	20771,1
12h	5722,7	2638,1	2525,2	891,8	6451,5	2210,3
24h	211,0	264,2	248,2	248,3	1459,6	573,0

Time	Ovary	±	Brain	±	Colon content	±
2h	17434,4	14743,0	73,6	86,8	212942,4	187554,1
6h	81051,7	53759,0	0,0	0,0	63818,0	19241,7
12h	20325,1	9423,8	9,1	15,8	79615,2	4627,6
24h	5349,7	5392,3	23,5	40,7	1757,4	1736,5

5

From the results of the biodistribution of PBRM-II in six tissues, it can be seen that PBRM-II was not significantly detected in brain tissue. This suggests that it cannot cross the blood-brain barrier. PBRM-II was present in five of the tissues analyzed (liver, kidney, uterus, ovary and in the colon content), but it did not accumulate, and its concentration decreased over time. Additionally, there was a low level of PBRM-II detected at 24h, suggesting a complete clearance of the compound after 48h. A greater accumulation in the ovary and uterine tissues was observed compared to some of the other tissues.

VI. Half-life (T_½) of PBRM-II and PBRM in human liver microsomes (HLM)

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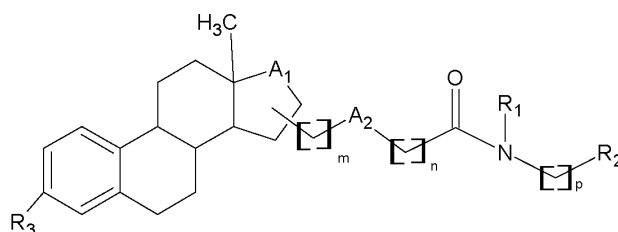
Incubations were performed in deep well plate. Potassium phosphate buffer (100 mM, pH 7.4, + 2 mM MgCl₂, 600 µL) containing human liver microsomes (0.53 mg protein/mL) was pre-incubated with PBRM-II (4 µM) or positive control (loperamide, 1 µM) in a water bath at a temperature set at 37°C for 10 min (N=1). The T0 was obtained by transferring 47.5 µL of the preincubated microsomes containing the compound in a new 96 well plate kept on ice and by quenching with 100 µL of (1:1) methanol/acetonitrile. After that, 2.5 µL of NADPH solution at 20 mM was added to the quenched T0 sample to keep the same ratios than in

20

- the incubations. Reactions were initiated by transferring 380 μL of the preincubated microsomes containing the compound in a new row of the 96 well plate and adding 20 μL of NADPH (final concentration 1 mM). Using the remaining volume of the preincubated microsomes containing the compound, reactions without NADPH were incubated to rule
- 5 out non-NADPH metabolism or chemical instability in the incubation buffer. Reactions without compound were also incubated for blank control. Reactions were terminated at each time point (15, 60, 120, 180 and 240 min) by taking 50 μL of incubation solution and quenching with 100 μL of (1:1) methanol/acetonitrile. The samples were centrifuged at 1500 rpm for 5 min, and the supernatant was transferred into a 96-well microplate before analysis
- 10 by UPLC-QToF-MS. The percent of PBRM-II remaining versus time is shown in Figure 5A. Figure 5B shows the Ln of percent of PBRM-II remaining versus time. From this graph the half-life ($T_{1/2}$) of PBRM-II at 4 μM in human liver microsomes (HLM) was calculated to be 210 minutes.
- 15 The same protocol as for PBRM-II was used to determine the half-life of PBRM in human liver microsomes (Figures 5C and 5D). The half-life of PBRM in human liver microsomes was calculated to be 103 min. Therefore, PBRM-II is more stable than PBRM in human liver microsomes.
- 20 While illustrative and presently preferred embodiment(s) of the invention have been described in detail hereinabove, it is to be understood that the inventive concepts may be otherwise variously embodied and employed and that the appended claims are intended to be construed to include such variations except insofar as limited by the prior art.

CLAIMS

1. A compound of formula (I), or a stereoisomer or salt thereof:



(I)

wherein:

A₁ is selected from C(O) and CHR_{Z1};

A₂ is selected from CH₂ and O;

R₁ is selected from the group consisting of: hydrogen, (C₁-C₅)alkyl optionally substituted with one or more Z substituents, (C₁-C₅)alkoxy optionally substituted with one or more Z substituents, (C₂-C₅)alkenyl optionally substituted with one or more Z substituents, (C₂-C₅)alkynyl optionally substituted with one or more Z substituents; (C₃-C₈)cycloalkyl, aryl, and heteroaryl;

R₂ is a heterocyclic aromatic ring having 5, 6 or 7 members selected from the group consisting of: CR_{Z2}, N, S, and O, provided that at least one of the members is N, S or O;

R₃ is (C₁-C₅)alkyl substituted with one or more Br, Br⁷⁶, I, I¹²³, I¹²⁴, or I¹³¹;

R_{Z1} is selected from OH, NR_{X1}R_{X2}, =N-OR_{X3}, halogen, (C₁-C₅)alkoxy, OC(O)(C₁-C₅)alkyl, and OSO₂NR_{X4}NR_{X5};

R_{Z2} is selected from hydrogen, OH, (C₁-C₁₀)alkyl optionally substituted with one or more Z substituents, and (C₁-C₁₀)alkoxy optionally substituted by one or more Z substituents;

Z is selected from halogen, OH, (C₁-C₅)alkyl, (C₁-C₅)haloalkyl, (C₁-C₅)alkoxy, (C₁-C₅)haloalkoxy, and NR_{X1}R_{X2};

R_{X1}, R_{X2}, R_{X4} and R_{X5} are the same or different and are selected from the group consisting of: hydrogen, (C₁-C₅)alkyl, (C₁-C₅)haloalkyl, (C₁-C₅)alkoxy, and (C₁-C₅)haloalkoxy;

R_{X3} is selected from H, and (C₁-C₅)alkyl;

m represents an integer value selected from 0 to 2;

n represents an integer value selected from 0 to 2;

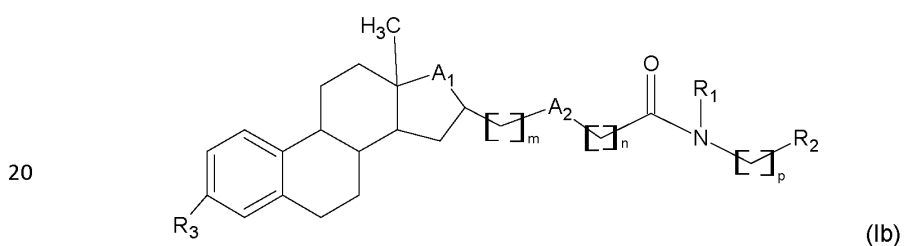
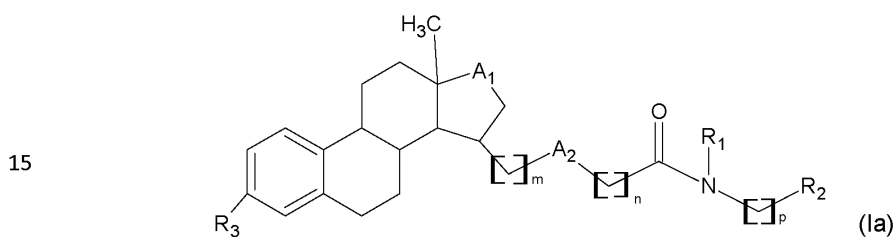
p represents an integer value selected from 0 to 5;

wherein:

- 5 aryl is an aromatic ring system comprising 5 or 6 CR_c members, wherein R_c is selected from H, halogen, cyano, nitro, (C₁-C₅)alkyl, (C₁-C₅)haloalkyl, -O-(C₁-C₅)alkyl, and -O-(C₁-C₅)haloalkyl; and

heteroaryl is an aromatic ring system comprising 5 or 6 members selected from the group consisting of: CR_d, O, N, NH, and S; wherein R_d is selected from H, halogen, cyano, nitro,
 10 (C₁-C₅)alkyl, (C₁-C₅)haloalkyl, -O-(C₁-C₅)alkyl, and -O-(C₁-C₅)haloalkyl.

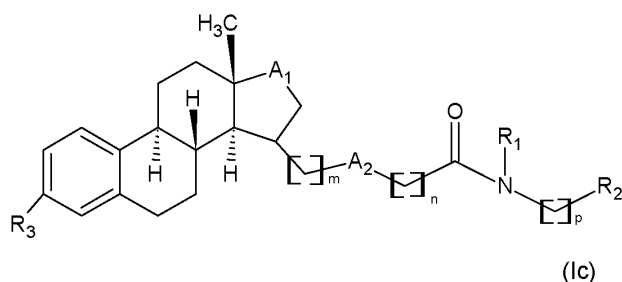
2. The compound of claim 1, which is one of formula (Ia) or (Ib):



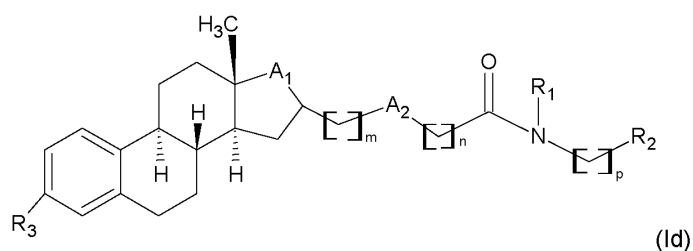
wherein R₁ to R₃, A₁, A₂, m, n, and p are as defined in claim 1.

3. The compound of claim 1 or 2, which is one of formula (Ic) or (Id):

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wherein R_1 to R_3 , A_1 , A_2 , m , n , and p are as defined in claim 1.

4. The compound of any one of claims 1 to 3, wherein A_1 represents $C(O)$ or $CHOH$.
5. The compound of any one of claims 1 to 4, wherein m is other than 0, and $A_2 = CH_2$.
6. The compound of claim 5, wherein m is 1, $A_2 = CH_2$ and n is 0.
7. The compound of any one of claims 1 to 4, wherein $m = 0$, and $A_2 = -O-$.
8. The compound of claim 7, wherein $m = 0$, and $A_2 = -O-$ and n is other than 0.
9. The compound of claim 8, wherein $n = 1$.
10. The compound of any one of claims 1 to 9, wherein R_1 is hydrogen.
11. The compound of any one of claims 1 to 10, wherein R_3 is bromoethyl.
12. The compound of any one of claims 1 to 11, wherein R_3 is 2-bromoethyl.
13. The compound of any one of claims 1 to 12, wherein R_2 is a heterocyclic aromatic ring having 5 or 6 members.
14. The compound of any one of claims 1 to 13, wherein R_2 is a heterocyclic aromatic ring having one or two heteroatoms.
15. The compound of claim 14, wherein the one or two heteroatoms are N or S.

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16. The compound of any one of claims 1 to 15, wherein p is 0 when R₂ represents a 5-membered heterocyclic aromatic ring.
17. The compound of any one of claims 1 to 15, wherein p is 1 when R₂ represents a 6-membered heterocyclic aromatic ring.
- 5 18. The compound of any one of claims 1 to 17, wherein R₂ is a thiazolyl ring wherein R_{Z2} is as defined in claim 1.
19. The compound of claim 18, wherein R_{Z2} is selected from H and (C₁-C₅)alkyl.
20. The compound of any one of claims 1-15 and 17, wherein R₂ represents a pyridinyl ring, wherein R_{Z2} is as defined in claim 1.
- 10 21. The compound of claim 20, wherein R_{Z2} is selected from H and (C₁-C₅)alkyl.
22. The compound of any one of claims 1 to 21, which is selected from the group consisting of:
- 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15β-yl]-N-(5-methyl-1,3-thiazol-2-yl)propenamide;
- 15 3-[3-(2-bromoethyl)-17β-hydroxyestra-1(10),2,4-trien-15β-yl]-N-(5-methyl-1,3-thiazol-2-yl)propenamide;
- 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-(5-methyl-1,3-thiazol-2-yl)propenamide;
- 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-(5-methyl-1,3-thiazol-2-yl)acetamide;
- 20 3-[3-(2-bromoethyl)-17β-hydroxyestra-1(10),2,4-trien-16β-yl]-N-(5-methyl-1,3-thiazol-2-yl)acetamide;
- 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]propenamide;
- 25 3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]acetamide;
- 3-[3-(2-bromoethyl)-17β-hydroxyestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]propenamide;
- 3-[3-(2-bromoethyl)-17β-hydroxyestra-1(10),2,4-trien-16β-yl]-N-[(pyridin-3-yl)methyl]acetamide;

3-[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15 β -yl]-N-[(pyridin-3-yl)methyl]propenamide;

3-[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-15 β -yl]-N-[(pyridin-3-yl)methyl]propenamide;

- 5 2-[[3-(2-bromoethyl)-17-oxoestra-1(10),2,4-trien-15 β -yl]oxy]-N-(5-methyl-1,3-thiazol-2-yl)acetamide; and

2-[[3-(2-bromoethyl)-17 β -hydroxyestra-1(10),2,4-trien-15 β -yl]oxy]-N-(5-methyl-1,3-thiazol-2-yl)acetamide;

or any salt or stereoisomer thereof.

- 10 **23.** A pharmaceutical composition comprising the compound as defined in any one of claims 1-22, and one or more pharmaceutically acceptable excipients or carriers.

24. A kit of parts comprising:

(i) the compound as defined in any one of claims 1-22 or the pharmaceutical composition of claim 23, and

- 15 (ii) a detectable label.

25. A method for inhibiting the 17 β -HSD1 enzyme in a subject, the method comprising the step of administering a therapeutically effective amount of the compound of formula (I) as defined in any one of claims 1-22 or the pharmaceutical composition as defined in claim 23 to a subject in need thereof.

- 20 **26.** A method for the treatment and/or prophylaxis of an estrogen-dependent disease or disorder, the method comprising the step of administering a therapeutically effective amount of the compound of formula (I) as defined in any one of claims 1-22 or the pharmaceutical composition as defined in claim 23, to a subject in need thereof.

27. The method of claim 25 or 26, wherein the subject suffers from a malign steroid dependent disease or disorder.

28. The method of claim 27, wherein the malign steroid dependent disease or disorder is a cancer.

29. The method of claim 25 or 26, wherein the subject suffers from a benign steroid dependent disease or disorder.

30. The method of claim 29, wherein the benign steroid dependent disease or disorder comprises endometriosis, uterine fibroids, uterine leiomyoma, adenomyosis, dysmenorrhea, menorrhagia, metrorrhagia, prostatic hyperplasia, urinary dysfunction, polycystic ovarian syndrome, lower urinary tract syndrome, multiple sclerosis, obesity, rheumatoid arthritis, colon cancer, tissue wounds, skin wrinkles or cataracts.
31. The method of any one of the claims 25 to 30, wherein the compound of formula (I) is administered in combination with one or more other therapeutic agents suitable in the treatment of steroid dependent diseases.
32. Use of the compound of formula (I) as defined in any one of claims 1-22 or the pharmaceutical composition as defined in claim 23 for inhibiting the 17 β -HSD1 enzyme in a subject.
33. Use the compound of formula (I) as defined in any one of claims 1-22 or the pharmaceutical composition as defined in claim 23, for the treatment and/or prophylaxis of an estrogen-dependent disease or disorder of a subject.
34. The use of claim 32 or 33, wherein the subject suffers from a malignant steroid dependent disease or disorder.
35. The use of claim 34, wherein the malignant steroid dependent disease or disorder is a cancer.
36. The use of claim 32 or 33, wherein the subject suffers from a benign steroid dependent disease or disorder.
37. The use of claim 36, wherein the benign steroid dependent disease or disorder comprises endometriosis, uterine fibroids, uterine leiomyoma, adenomyosis, dysmenorrhea, menorrhagia, metrorrhagia, prostatic hyperplasia, urinary dysfunction, polycystic ovarian syndrome, lower urinary tract syndrome, multiple sclerosis, obesity, rheumatoid arthritis, colon cancer, tissue wounds, skin wrinkles or cataracts.
38. The use of any one of the claims 32 to 37, in combination with one or more other therapeutic agents suitable in the treatment of steroid dependent diseases.